A Summary of Current Program 7/1/66

and Preliminary Report of Progress

for 7/1/65 to 6/30/66

NORTHERN

UTILIZATION RESEARCH AND DEVELOPMENT

DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

This progress report is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1965, and June 30, 1966. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Northern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Peoria, Illinois.

UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

The Northern Utilization Research and Development Division, located at Peoria, Illinois, is one of four research divisions of the Agricultural Research Service concerned with the development of basic knowledge of chemical composition and physical properties of farm commodities and with the application of this knowledge to the development of new or improved products and processing technology that will enhance utilization of these commodities. The other Utilization Research and Development Divisions are the Eastern at Philadelphia, Pennsylvania, the Southern at New Orleans, Louisiana, and the Western at Albany, California.

The need and importance of utilization research on farm commodities arise from the fact that the farmer is not organized to carry on modern scientific research to maintain old, and create new, markets for his products. The Northern Division is responsible for the Department's utilization research on corn, sorghum, soybeans, flax, crambe, and new crops. Its research on wheat emphasizes industrial utilization and milling technology, and that on forages is limited to a search for the cause of toxicity occasionally displayed by tall fescue grass. Responsibility for research on food and feed utilization of wheat and for the Department's primary utilization research program on forages is assigned to the Western Division.

The scientific research effort at the Northern Division amounts to approximately 195 scientist man-years. In addition, the Division supervises domestic research contracts equivalent to 35.1 scientist man-years and grants equivalent to 13.9 scientist man-years, and sponsors a comprehensive program of research comprising 45 PL 480 grants to foreign institutions.

In this report, utilization research of the Northern Division is discussed under the 17 Area Headings shown in the Table of Contents. For each area, a description of the current research program is provided, including domestic research contracts and grants and sponsorship of related research performed abroad under grants of Public Law 480 funds. A preliminary report of progress and a list of publications is given for each area for the period July 1, 1965, through June 30, 1966.

As a step toward implementation of the recommendations for a National Program of Research for Agriculture made jointly by the Association of State Universities and Land Grant Colleges and the USDA, a section has been added to each of the Areas in this report. It comprises a list of the related publications of the State Agricultural Experiment Stations in addition to those heretofore reported covering the results of USDA and cooperative research. In future years, it is anticipated that information will be available to permit reporting of achievements resulting from State research in a format comparable to the present reporting of the USDA and cooperative research.

For each area, the size of the related program maintained by State Experiment Stations is given. In addition to examples of recent utilization research accomplishments of the Northern Division, examples of achievements by the State Stations are provided.

RECENT ACCOMPLISHMENTS OF THE NORTHERN DIVISION

New use for starch in urethane foams. Department scientists have developed a new process for making polyethers from starch for use in the production of rigid urethane foam. Rigid urethane foam from all sources is now being sold at a rate of 100 million pounds per year. Estimates are for production of 280 million pounds by 1969. The increasing demand is due to its high insulation capacity, which is about twice that of non-urethane foams. The construction, transportation, and appliance industries are the major consumers of rigid foams. The amount of starch-derived polyether used in making 100 pounds of foam requires 13-15 pounds of starch.

Studies conducted under a research contract have proven that (1) polyethers can be prepared successfully in 1,000-pound lots; (2) polyethers can be used in conventional foam formulations; and (3) foams can be produced in blocks or formed in place in deep panels by commercial foam machines. Cost estimates indicate that a plant to produce 10 million pounds per year of polyethers from corn starch at a capital investment of less than $470,000 could operate to sell the polyethers at a price favorable for polyethers derived from starch.

A complete technical report on its production, use, and cost has been supplied to more than 125 industrial and technical inquirers. Up to midyear of 1966, two chemical companies have been licensed to use the Department's patent. Many companies have made trials of the process on a scale of up to 100 pounds and have reported confirmation of our results. One company is reported to have purchased two carlots of starch for large-scale evaluations. The outlook is most encouraging for a significantly large new outlet for cereal starch in this application.

Soy flour process for local production in developing countries. A favored approach for alleviating the food shortages in the developing nations is to provide low-cost protein foods based on crops that can be grown locally. For this purpose, Department engineers have devised a simple method for hand production of full-fat soy flour. The process is designed for use in foreign villages where skilled labor, electric power, and steam are not available.

In this process, both equipment and fuel requirements are minimal and hand labor is used in place of mechanical power. The total cost of equipment is estimated to be below $200 for producing about 300 pounds of full-fat soy flour per 8-hour day. This production, which can be accomplished by five men, is sufficient to supply 35 grams of protein per day (1/2 the minimum daily protein requirement) to more than 1,600 adults. The remaining

step to be taken is on-site demonstration of this simple flour process under the environmental conditions of a specified developing country.

Linseed oil protection for concrete. Use of salt for ice and snow removal has caused increasing damage to concrete highways under winter conditions. Department scientists cooperating with industrial, Federal, State, and local groups have demonstrated that thin coatings of linseed oil help protect concrete against scaling and spalling. The oil may be applied as a solution of oil-mineral spirits or as a Department-developed oil-water emulsion. Application by spraying is usually most economical, but alternate methods may be used.

Concrete varies so much in composition and performance, and roads are subject to such different uses that obtaining conclusive technical information requires long periods of time. However, results already have been positive enough that the State of Illinois recently specified the use of linseed oil on all new concrete roads instead of only on bridges as previously required. Country-wide adoption would result in greatly increased consumption of linseed oil. Flaxseed from which linseed oil is obtained is an important crop in the States of Minnesota, North Dakota, South Dakota, and Texas.

Industry uses acid-modified cereal flours. Department scientists and engineers have developed a process for the chemical modification of cereal flours for use as surface-sizing agents for paper. The acid-modified flour, called AMF for short, was prepared in lots of several hundred pounds for a number of trials on laboratory, semicommercial, and commercial paper machines. Its performance on paper machines and the quality of the finished paper are about the same as with high-grade commercial modified starches. Encouraged by these findings, a wheat flour milling company with diminishing outlets for soft wheat flour, in cooperation with the Department and a commercial paper mill, conducted a trial in which laminated fiberboard was surface sized, the machine running at a normal rate of 75 tons per day. The finished fiberboard met the mill specifications. The cooperating milling company is planning to install a small plant to make AMF.

Four large milling companies are selling acid-modified corn or sorghum flour for use in paper sizing and gypsum board. All are using either the Department's process or a modification of it. Cost of the modified flours is favorable. No figures are available for current sales of AMF. The paper industry uses 600 million pounds of other corn and sorghum products per year for surface sizing and the gypsum board industry uses over 50 million pounds. Increased use of AMF is expected to develop largely in the coarse paper and box paper industry. The outlook is good, particularly in regions where flour and paper mills are in close proximity. Other producers of flour both in the U. S. and abroad are much interested in making AMF.

Lower cost sirup from cereal grains. Industry has adopted and is now extensively using the process developed by Department scientists for the conversion of the starch in whole cereal grains to low-cost sirup by the use of bacterial and fungal enzymes. A key step in this development was the discovery of a microorganism which gave a higher yield of the desired enzyme than previously available while reducing the level of undesired enzymes. The sirups obtained by this enzymatic treatment make it possible for cereal grain products to compete with imported molasses used in the fermentative production of chemicals and antibiotics.

The Department has a continuing research program to discover enzyme sources; to develop analytical, isolation, and purification techniques for new enzymes; and to use enzymes for the conversion of starch and its derived products into useful food and chemical products. Adoption of the USDA developments has and could continue to lead to expanded markets for grains.

USDA's wheat gluten process commercialized. A continuous process was developed by Department scientists for separating wheat flour into starch and food-grade gluten. Five U. S. industrial installations are using this process, or modifications of it, on over 265 million pounds of flour annually. The process is simple in operation, and no addition of chemicals is required. A new plant now is nearing completion in the Pacific Northwest which will use second clears flour as the raw material. This plant will take advantage of regional markets and raw material supplies. The installation will include facilities to produce different grades of starch and food-grade gluten. Facilities for conversion of starch into sirup will also be installed. Gluten has many important uses including increasing the protein content of flours for baking and increasing the protein level of breakfast cereals. Wheat starch has broad use in foods and such industrial outlets as paper additives, wallpaper pastes, and plywood glue additives. Department scientists have been extensively consulted on the physical, chemical, and mechanical properties of wheat flour and its processing as a guide for design and installation of the new plant.

New information on seed protein content and amino acid composition. The most comprehensive survey ever made of seeds as protein sources has been conducted by Department scientists. Of seed from the 4,000 plant species investigated, 475 had more than 35 percent protein. Amino acid analyses by precise modern procedures were completed on 379 species. This is over four times the total number of species for which reliable amino acid values were available prior to 1957 when this survey program was started. Seed protein of some species contained amino acids in relative proportions that suggest good nutritional balance. Others, while not themselves balanced, were rich in amino acids present in suboptimal amounts in diets based primarily on cereal grains. The data obtained offer a basis for suggesting species for field trials, animal tests, and for adaptability to the agricultural economy of particular regions of the world. From such considerations and experiments, promising new feed and food crops may result.

Improved gas analyzer speeds analysis of fats. A major problem in basic research on vegetable fats is the analysis of the raw materials and reaction products. Department scientists have devised a novel, rapid, and accurate method for analyzing the structure of fats. The method is 40 times faster than previous procedures and gives equivalent accuracy of results. The procedure combines the use of ozone and a soldering gun to supply heat for decomposing a sample of fat and a gas analyzer to determine the decomposition products. This unique combination has facilitated research on the flavor stability of soybean oil and on the quality of the oil's derived products: shortening, margarine, and salad and cooking oils. The method also should be helpful in determining the fate of nutritionally important fats in processing and use.

RECENT ACCOMPLISHMENTS OF STATE AGRICULTURAL EXPERIMENT STATIONS

Fatty acids of wheat. Using gas chromatographic analysis, Minnesota biochemists have extended the knowledge of wheat lipids and comprehensively accounted for the distribution of fatty acids between free and bound lipids present in the milled fractions: bran, germ, and endosperm. Since bound lipids include the phospho- and glycolipids, one can predict that their fatty acid content is less than that for glycerides present in free lipids. In fact, the fatty acid content of free lipids is about twice that of bound lipids in bran and 1.5-fold greater in straight-grade flour. Linoleic acid is the predominant fatty acid in all fractions. Precision of the analytical method permitted an accounting for all the minor fatty acids in the range from 11-carbon to 22-carbon fatty acid molecules. Of the five major fatty acids in wheat lipids, their decreasing order of abundance is linoleic, palmitic, oleic, linolenic, and stearic; however, the distribution of the individual fatty acids between free and bound lipids is different for bran, shorts, and flour. Current interest in the intake of saturated fatty acids from a nutritional view is reflected in this work.

Improved corn proteins. To overcome the nutritional deficiencies which corn proteins possess, it has been a common animal feed practice to add enriching dietary supplements which compensate for the two limiting amino acids, lysine and tryptophane. Scientists at the Indiana Station have discovered in corn that mutant genes, such as opaque-2 and floury-2, change the protein composition and amino acid pattern of the kernel. A new high-lysine corn variety, opaque-2, containing 100 percent more lysine and 69 percent more tryptophane than normal seed, is being tested for high commercial yield and for nutritive value to swine, poultry, and humans. Another high-lysine corn, floury-2, contains both increased tryptophane and methionine levels. Improved protein quality in the new genotypes results from boosting the glutelin content, lysine-rich, and decreasing the zein level. Protein utilization of these genotypes for human nutrition offers distinct promise and has an important bearing on world food supplies.

Chemicals in corn control resistance to the corn borer. Although larvae of the European corn borer have been responsible for losses to corn crops in past years amounting to over $100 million per year, certain plant varieties have been found which are resistant to the corn borer. Recent basic research at the Iowa Agricultural Experiment Station has shown that a chemical substance present in the resistant strains of corn is a powerful feeding deterrent to the corn borer. The substance, isolated in pure form from corn seedlings and identified as a cyclic member of the hydroxamic acid family, is capable of killing the corn borer when fed at a level equivalent to its concentration in resistant inbred lines of corn. The possibility exists of field treating other varieties of corn with this substance to protect plants from corn borer ravages. Moreover, this finding may stimulate a search for chemical factors in natural resistance to other pests. A rapid method of analysis for specific hydroxamic acids in corn is being developed and will be of value to the plant breeder in predicting the corn borer resistance of new crosses.

Control in protein synthesis. All animals and plants are shaped into images of their ancestors by directives contained in chemical messages. Precision manufacture of specific proteins in living beings is controlled by complex substances known as nucleic acids which reside in the cells. The complete chemical make-up of a ribonucleic acid (RNA) has become known through the efforts of Dr. R. W. Holley and his seven-man team at Cornell University. This important biochemical accomplishment resulted from a joint USDA and Cornell research support program over a 3-year period. The way has been opened for determining the structure of other nucleic acids involved in the pathways of inheritance. This is the first nucleic acid, alanine transfer RNA, whose structure is known and the first link in an intricate chain of genetic biochemistry.

Polymers from glucose derivatives. A new method for making water-soluble glucose polymers has been developed by station workers. Applicable to sugar 1,2-ketals derived from low-cost corn sugar, the experimentally mild conditions of the process result in highly branched polymers. Regarded as low-molecular-weight gums, the products have potential use in foods, pharmaceuticals, adhesives and textile sizing. Isopropylidene derivatives of glucofuranose, when treated with a Lewis acid, such as boron fluoride, react to eliminate acetone and produce condensation products with molecular weights as high as 13,000. Sugar ring expansion occurs during the treatment, since up to 95 percent of the polymer units are 6-member D-glucopyranoside units. There is no need for high vacuum equipment to eliminate the acetone in this simple process for polymerizing sugars.

"Milk" from wheat flour. Indiana scientists (partially supported by ARS-USDA) have prepared a milk-like product from wheat flour. Transferring a high level of protein from the flour to the milk-like product was the difficult problem. Using a process similar to digestion, the scientists were able to recover 85 to 90 percent of the wheat flour protein and to develop a dry high-protein concentrate which is soluble in water.

Nutritional properties of the product are similar to non-fat dry milk. When mixed with water, it looks like milk and has a bland flavor. The concentrate could also be added to soups and other prepared foods to improve their nutritional properties. The process was developed in search for a low-cost, palatable, milk-like product to improve human nutrition in distressed areas and underdeveloped countries.

Leaf protein potential as foodstuff. Leaf protein concentrates from several plant species were extracted from green leaves by mechanical means and analyzed for their amino acid and fatty acid composition by Wisconsin station researchers. Nutritive value was estimated by *in vitro* enzymatic digestion. On an amino acid compositional basis, leaf protein concentrate should be a well-balanced source of dietary protein, if supplemented with synthetic methionine. The concentrates contained from 3 to 8 percent fatty acids, more than 75 to 80 percent of which were linolenic, palmitic, and linoleic acids. Digestibility was estimated from the total amount of amino acids released by pepsin followed by pancreatin hydrolysis. The leaf proteins compared favorably with high-quality proteins. Results indicate that plant leaves may be an important source of food for human consumption, especially in the developing countries. Recently, leaf protein concentrates were prepared by spray-drying the juice expressed from fresh alfalfa and pea vines. The concentrate was a green powder of low fiber content which could be fed to nonruminant animals. A product which might be further processed for human consumption was prepared by extraction of the spray-dried preparation with ethanol. On the basis of amino acid analyses and the pepsin pancreatin digest indices, the protein could potentially be equal to or superior than high-protein feedstuffs now available. The vitamin content of the spray-dried preparation was higher than that found in commercial dehydrated alfalfa products.

AREA NO. 1: CORN UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Almost 2.5 billion pounds of cereal starches and flours are used annually in the U. S. for industrial purposes. Corn is the source of most of these products. Industrial outlets for starches and flours are, however, constantly threatened by synthetic products derived from non-agricultural sources. Maintenance of the present and future competitive position of corn starch and flour in industrial markets requires a continuing program of basic and applied research.

The most promising outlets for new and improved industrial products derived from corn include the paper industry, industrial chemicals, adhesives, protective coatings, plastics, elastomers, and thickening agents. The greatest opportunity exists in the manufacture of paper and paperboard products, the U. S. production of which is 43 million tons per year and growing at the rate of 3.3 percent per year as compared to GNP growth of 3.4 percent and population growth rate of 1.7 percent per year. Use of starches in paper doubled between 1950 and 1963 from 552 million pounds to 1.1 billion pounds. This growth rate is 5.6 percent per year. The average amount used per ton of paper product has increased from 19 to 29 pounds. This favorable picture stems from research conducted in the past. To maintain or, more desirably, to increase the utilization of corn starch and flour in competition with synthetics, new concepts must be evolved that relate chemical modification of starch with specific properties imparted to paper products. Technology must be developed to establish optimum procedures for industrial use of promising products currently under study such as starch xanthates, starch graft copolymers, cyanoethylated and sulfated starches, and the new starch from high-amylose corn.

At 5 to 6 cents per pound, corn starch is an attractive raw material for the manufacture of products that can find outlets in the multibillion-pound annual market for industrial chemicals. When fermentative, rather than conventional chemical, conversion is applicable, even cheaper sources of starch such as flour and ground whole grain can be used as the raw material. Over a billion pounds of corn sugar is used annually in the manufacture of such well-known industrial chemicals as sorbitol, mannitol, citric and gluconic acids, and methyl glucoside. Promising leads requiring research to ensure successful future developments include nitrogen, sulfur, and unsaturated derivatives of starch, vinyl glucosides, industrial enzymes, and enzymatic starch conversion products such as oligosaccharides, polyols, and glucosides.

Adhesives represent a field long dominated by starch, which accounts for nearly half of the annual 2-billion-pound market for these products. However, competition by synthetic resins is especially vigorous and effective because of the specialized properties required to achieve increased

production of products like corrugated container board on automatic machinery. The overall growth rate of adhesive consumption is almost 7 percent per year. Since starch is usually cheaper than synthetic adhesive resins, prospects are good for meeting the competition through research designed to improve viscosity properties, bond strength, tack, and drying time of starch-derived adhesives.

The remaining outlets--coatings, plastics, elastomers, and thickeners--represent a multibillion-pound market in which starch-derived products having suitable properties should find ready acceptance. Microbial polysaccharides, starch graft copolymers, and urethane foams from starch-derived polyols typify the products that result from research on corn starch and flour.

Research oriented specifically toward particular industrial applications of final products must be founded on a vigorous and wide-ranging program of basic and exploratory investigations. Such studies lead to the discovery of new concepts, principles, and reactions that are the source of new processes and products for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of corn, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of corn totals 73.0 scientist man-years. Of this number, 12.2 are devoted to chemical composition, physical properties and structure; 25.2 to chemical and physical investigations to improve products; 21.4 to microbiology and fermentation; and 14.2 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (9.9 scientist man-years) involves study of starch, amylose, amylopectin, proteins, and lipids of corn. Much of the work is related to problems pertinent to high-amylose corn. A research contract at Arizona State University, Tempe, Arizona, for basic research on reactions of "V" amylose has been completed. Grants (2.3 scientist man-years) have been made to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for basic studies on variations in starch granules of genetically different corn samples; to Princeton University, Princeton, New Jersey, for basic research on the relationship of viscoelastic properties of amylose film to structure and function of plasticizers;

to Iowa State University, Ames, Iowa, for basic research* on heat, mass, and momentum transport of cereal starches and flours; to Purdue Research Foundation, Lafayette, Indiana, for research* on the effects of disulfide bond cleavage on the structure of corn and wheat endosperm proteins; and to Arizona State University, Tempe, Arizona, for basic investigations of the helical structure of amylose.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (19.1 scientist man-years) is directed to wide-ranging study of the chemical reactions of starch with the objective of discovering new chemical products and processes having potential for industrial use. During the year, one phase of this work involving study of possible means for preparing amino acid derivatives of starch was completed and replaced by research on synthesis of halogen derivatives of starch. Research contracts (2.6 scientist man-years*) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of acetylene with methyl glucoside; with The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media; to the University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides; with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers; and with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper. Contract research was completed by Ohio State University, Columbus, Ohio, on synthesis of amino derivatives of starch and by the University of Arizona Agricultural Experiment Station, Tucson, Arizona, on the reaction of starch with mercaptans. Grants (3.5 scientist man-years*) have been made to Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; to Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; to Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur; and to the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides.

Research on microbiology and fermentation conducted at Peoria, Illinois, (21.4 scientist man-years) includes studies on the use of microorganisms to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. A large collection of pure cultures of industrially and agriculturally important microorganisms is maintained. The Pioneering Laboratory for Microbiological

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*Work covers more than one commodity; only effort allocated to corn is included in total.

Chemistry conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (1.0 scientist man-year*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation* of morphological changes involved in sporulation; and at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective. Contract research at the University of Illinois, Urbana, Illinois, on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens has been completed. Grants (2.2 scientist man-years*) have been made to Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes; to Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; to Iowa State University, Ames, Iowa, for investigation* of bacterial amylases and their action patterns; to the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; and to the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch.

Research conducted at Peoria, Illinois, on technology - process and product development (9.2 scientist man-years) is concerned with detailed study and evaluation of starch derivatives having definite potential for industrial utilization and of processes for making them. Research contracts (5.0 scientist man-years*) are in effect with Stanford Research Institute, Menlo Park, California, for process development of selected starch graft copolymers; with Battelle Memorial Institute, Columbus, Ohio, for developmental research on starch and other cereal grain xanthides and for studies on starch derivatives for use as colloids in water-emulsion paints; with Western Michigan University, Kalamazoo, Michigan, for evaluation of modified cyanoethylated starches for applications in paper; with Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations on the use of starch glycosides in coatings and plastics; and with University of Akron, Akron, Ohio, for evaluation of starch and starch derivatives as reinforcing agents for natural and synthetic rubber. During the year, contract research on evaluation of starch polyol urethane foams was completed by Archer Daniels Midland Company, Minneapolis, Minnesota.

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*Work covers more than one commodity; only effort allocated to corn is included in total.

The Department also sponsors research on cereal starches, conducted by foreign institutions under grants of PL 480 funds.* Research on chemical composition, physical properties and structure involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); to the University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968); and to "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967). During the year, research on corn zein was completed at the National Institute of Agronomic Research, Paris, France.

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); Academy of Sciences and Chemical Institute "Boris Kidric," Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); and Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); and to the Institute for Fibres and Forest Products Research, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968). During the year, research was completed on fatty chemical derivatives of starch dextrins at the Institute of Industrial Chemistry, Bologna, Italy, and on changes induced in starch by gamma-irradiation at the National Institute of Agronomic Research, Paris, France.

Research on microbiology and fermentation involves grants to the University of Milan, Milan, Italy, for basic studies on the metabolic pathway to 5-ketogluconic acid in Acetobacter species (5 years, 1961-1966); University of Allahabad, Allahabad, India, for collection of new Mucorales species (5 years, 1961-1966), and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India

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*Effort prorated among corn, wheat, and grain sorghum.

to find new accessions for the ARS Culture Collection (5 years, 1965-1970); to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969), and of mevalonic acid (3 years, 1965-1968); to the Institute of Biological Chemistry, University of Rome, Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966); to the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and to the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. High-amylose (HA) corn development. During the reporting year 12,517 amylose analyses were reported to cooperating corn breeders. To date, a total of 17 samples containing at least 85 percent apparent amylose have been found. Techniques for single-kernel analysis based on excision of only a small portion of the kernel were improved, and about 300 individual kernels were successfully analyzed by this method. Despite the damage caused by sampling, these kernels showed a high percentage of germination. Reports from breeders and millers indicate a sharply increased industrial demand for HA starch, presumably for uses other than coating glass fibers. It is thought that much of the increase is in response to demand from the paper industry. Another interesting report is that demand for Class 5 starch (50-60 percent amylose) continues despite availability of HA corn having higher amylose content. Apparently there is a real possibility for marketing a series of HA starches varying in amylose content to fill specific needs of industry.

2. Genetic control of starch properties. Research under the grant to the University of Nebraska showed clearly that specific recessive genes control properties of starch such as water absorption, viscosity, gelatinization temperature, and enzyme susceptibility. Starch associated with *wx* gene shows the highest water absorption, that with *ae* gene the lowest. The *su*$_2$ gene control contributes low viscosity and a low temperature of initial starch gelatinization. The *ae* starches showed the lowest enzyme digestibility; digestibility was reduced by *ae* gene even when occurring in combination with other recessive genes which normally result in starch of high digestibility. A combination of *su*$_2$ and *wx* resulted in a starch more

rapidly digested than starch from su2 or wx acting singly. This study could provide the basis for breeding varieties of corn containing starch with solution properties meeting specific requirements.

3. Chemical studies on corn components. Basic investigations indicated that lithium salts, urea, and guanidinium salts disperse starch granules by production of a surrounding aqueous medium having a dielectric constant greater than that of water. "R-enzyme" as obtained from potato tubers was separated into two components. Approximate molecular weights of the components were 20,000 and 1,500. The high-molecular-weight component shows α-amylase activity, i.e., it hydrolyzes both amylose and amylopectin. The small molecule increases iodine affinity of the substrate apparently by increasing turbidity of the solution. Molybdate ion prevented precipitation of this small molecule and therefore repressed this reaction.

In the contract research at Arizona State University, now completed, more precise study of the reversible dehydration of V-amylose hydrate gave a value for ΔH of 10.4 kcal/mol as compared to 9.0 kcal reported earlier. Alcohols dehydrated V-amylose hydrate with apparent occupation of the interior of the helix through interaction with glycosidic oxygens. On the other hand, water, ammonia, and formaldehyde appeared to interact with hydroxyl groups on the exterior of the helix. Further study showed that interaction of V-amylose and ammonia was not completely reversible. Absorption of ammonia was greatest at high pressures and low temperatures. More detailed investigation of selective reactions between V-amylose and small molecules will be carried out under a grant to Arizona State University.

Initial studies under the grant to Purdue University for research on disulfide bond cleavage were directed to developing methods necessary for the evaluation of the effect of alkali on proteins. A procedure for amide nitrogen analysis was developed wherein cystine residues are first reduced with bisulfite to eliminate interference of cystine during alkaline hydrolysis of amides. Conditions for treatment of tryptophan with sodium nitrite were optimized to render the Spies and Chambers colorimetric determination of tryptophan in protein more precise. Proteins of both corn and wheat will be studied under this grant.

Studies on zein protein under a PL 480 grant to the National Institute of Agronomic Research, Paris, France, have been completed. Zein was found to be complex. Although amino acid analyses indicate the presence of only two principal protein components, an array of heterogeneous fractions arises from the association of these proteins in various combinations with relatively small peptide chains and with variable amounts of pigment. The amount and type of the various protein complexes depends on conditions under which protein is isolated. Thus, the resolution of zein into well-defined and separated protein fractions is a difficult task. Mild conditions of purification are required to obtain reproducible experimental

results. Alkali treatment, such as is used in commercial zein preparation, results in a stable protein-pigment complex that owes its stability to the blocking of protein sulfhydryl groups.

At the University of London, London, England, under a PL 480 grant for research on starch- and glycogen-debranching enzymes, examination of the carbohydrase enzymes in sweet corn revealed an apparently new transferase that can transfer α-1,6 linked maltose units from one pentasaccharide to another. A new enzymatic method has been developed for obtaining the degree of branching and other structural analyses of amylopectins and glycogens.

4. Physical studies on corn components. Nuclear magnetic resonance studies have been undertaken to obtain characterizing information on amino acids and vinyl derivatives thereof and to elucidate effects on the chelating properties of ramulosin (a grain fermentation product) induced by halogen substitution. Infrared spectra of lipids isolated from starch fractions indicate that these lipids are free fatty acids.

Studies on use of dimethyl sulfoxide (DMSO) solutions of amylose for film-casting showed that DMSO-complexed films could be stretched to give oriented X-ray fiber diagrams for use in determining crystal structure. When converted to B-type structure, the DMSO-complexed film exhibited remarkably improved flexibility in comparison to films cast from butanol-water. Tensile strengths, however, were comparable (ca. 8,600 lbs./sq. in.). The number of double folds at 16 percent R.H. was used as a measure of brittleness. For films 1.5 mils in thickness the number of double folds observed was 1,000 for amorphous amylose, and 320 and 105 for crystalline "B"-amylose from DMSO and from water, respectively. V-amylose films were brittle at 16 percent R.H.

Under a grant at Princeton University, a number of basic properties of films from HA starch were measured, including modulus-temperature relationship, stress relaxation behavior, glass transition temperature, and glassy and rubbery moduli. These qualities were related to such factors as percentage of amylose in the starch and presence of plasticizers.

Research has been initiated at Iowa State University under a grant for basic investigations on heat, mass, and momentum transport in cereal starches and flours, but significant results have not yet been obtained.

At the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, IR and NMR investigations on model compounds and on amylose dissolved in DMSO have established an intramolecular hydrogen bond in amylose between specific hydroxyl groups of adjacent glucose residues. Such a bond not only serves to stabilize the helical form but also has an important bearing on the reactivity of amylose. This work is being conducted under a PL 480 grant.

In studies under a PL 480 grant to the University of Osaka Prefecture, Sakai, Japan, scientists are investigating possibilities of polarography of carbonyl groups as a means of analysis of starch and derivatives. They have succeeded in converting glucose to a polarographically active form, but the method of derivitization is not applicable to starches.

5. Chemical composition of corn grain. Compositional studies on corn are relevant to its industrial utilization. Results are reported under Area 3, subheading A.

B. Chemical and Physical Investigations to Improve Products

1. Reactions of maltose and glucose. Investigation of amide complexing ability revealed that in aprotic solvents isolatable complexes with urea were formed by glucose, maltose, and ribose. The complexes dissociate in aqueous and alcoholic media. N-substituted ureas do not complex. Continuing investigation of this phenomenon showed that in addition to urea, ethanol, dimethyl formamide, dimethyl sulfoxide, 2-oxazolidone, and hexamethyl phosphoric triamide associate with anhydrous maltose to form stable, isolatable molecular complexes. However, only urea formed an isolatable complex with glucose. The α- and β-anomers of D-glucose pentaacetate were found to form a 60:40 constant composition mixture that has a nearly constant melting point and optical rotation. This mixture crystallizes from various solvents even when different ratios of the components are present. It can also be recrystallized from various solvents without changing its properties. A simple method was developed for selective deacetylation (at C-1) of glucose and maltose acetates on silica gel. The structure of a third caramel-flavored compound discovered in studies of model sugar-amine browning reactions has been completely established to be 4-hydroxy-2,5-dimethyl-3(2H)-furanose.

2. Reaction of carbohydrates with mercaptans. Contract research at the University of Arizona on the reaction of mercaptans and carbohydrates has been completed. The most interesting product obtained during the study was the polymer from glucose and 1,10-decanedithiol. Final conclusions are that the best polymers are obtained in hydrogen fluoride (14-50 percent) catalyzed reactions in dioxane at 0-24° C. Dialdehyde starch did not react with the dithiol under these conditions but did yield a highly cross-linked, colored product if the reaction was run in liquid hydrogen fluoride. A variety of modified polymers was obtained by oxidation, acetylation, methylation, and xanthation of the glucose-decanedithiol reaction product. Glucose polythioacetals show some promise as adhesives. A covering patent application has therefore been filed.

3. New derivatives of starch and related carbohydrates. In final phases of research to explore possible methods of synthesizing amino acid derivatives of starch, the reaction of sulfur monochloride with carbohydrates was shown to yield complex mixtures of products containing both chlorine

and sulfur. No successful method was found for separating these mixtures; hence, the reaction is not a practical means for replacing hydroxyl groups of starch or glucose with chlorine. Because halogen derivatives of carbohydrates should be versatile intermediates and might prove to have potential for industrial use in synthesis and for other purposes, a study to find satisfactory methods of preparation of these derivatives has been undertaken.

Research under a grant to Ohio State University showed that 2,3-unsaturated glucose and mannose derivatives could be prepared by elimination reactions based on sulfonic esters (for trans glucose hydroxyls) or thionocarbonates (for cis mannose hydroxyls). Overall yields were 35-50 percent. Thiol derivatives and glucosyl sulfenyl halides could be added to olefinic bonds in sugar molecules. Specific wavelengths of UV light were shown to promote reactions at a sulfur atom in organic molecules without inducing side reactions at oxygen atoms.

Under a second grant to Ohio State University, tetrahydropyran-2-yl derivatives of amylose, amylopectin, and starch were prepared. Also, a series of acetals with various degrees of substitution were prepared from starch and methyl vinyl ether. These acetals were more labile to acid than were the tetrahydropyranyl derivatives. Vinyl ethers were found to exhibit differences in their reactivity toward starch.

At Purdue University, studies under a research grant showed that oxidation of penta-O-acetyl-α-D-glucothiopyranose with sodium metaperiodate yielded a crystalline sulfoxide whereas oxidation with peracetic acid formed a crystalline sulfone.

Contract research at Ohio State University on synthesis of amino derivatives of sugars has been completed. In final phases of the work, 3-amino-3-deoxy amylose was successfully synthesized and its structure was proven by degradation. Also, the corresponding 2-amino derivative was prepared, but time did not permit confirmation of its structure. Basic discoveries made during this work are being exploited under a grant for research on amination of starch with low-cost reagents.

In research under a PL 480 grant to the Plastics Research Institute TNO, Delft, The Netherlands, the preparation of sodium, potassium, lithium, calcium, barium, and magnesium alkoxides of starch by reaction of starch with the corresponding methoxides was studied in detail. Conditions were established for achieving wide ranges of substitution.

Research on fatty acid and fatty amine derivatives of dextrins has been completed by the Institute of Industrial Chemistry, Bologna, Italy. Various surface-active hydrophobic fatty acid esters and hydrophilic fatty amino dextrins were prepared and characterized. Industrial testing in Italy indicated possible uses for the esters as additives to printing inks, as agents for waterproofing paper, and as stabilizers for emulsions. The

amino dextrins promoted adhesion of active compounds to plant surfaces in agricultural spray emulsions. This research was conducted under a PL 480 grant.

4. Graft copolymers. Basic studies showed that the degree of swelling of starch granules strongly influenced the composition of the product obtained in ceric-ion-catalyzed grafting of acrylonitrile to starch. Variation of this parameter provides a further means for modifying properties of graft copolymers. Indeed, for at least one type of starch substrate, a satisfactory reaction requires avoidance of gelatinization.

Saponified graft copolymers of starch and methyl acrylate or acrylonitrile were found to have very high viscosities in solution. Both types of hydrolyzed graft copolymers showed good tolerance to salts. Cupric chloride was found to give the most favorable results when used as a chain modifier in the ceric-ion-catalyzed grafting of acrylonitrile to starch.

In contract research at Stanford Research Institute, studies to determine optimum conditions for grafting the following monomers to starch were completed: vinyl chloride; styrene; methyl methacrylate; vinyl acetate; and methyl, ethyl, and butyl acrylates. Good add-on and minimum formation of homopolymer were achieved for all except vinyl acetate and chloride, which gave no significant grafting under any conditions tested. Rate constants for the decay of free radicals were determined and activation energies for grafting were evaluated. Rate equations were developed for grafting of several monomers. (Pilot-plant studies on graft copolymers are reported under subheading 1-D-3.)

Under a PL 480 grant to the Hebrew University, Jerusalem, Israel, scientists have investigated the effects of reaction variables on length and frequency of grafts, monomer add-on, and product yield in the anionic grafting of ethylene and propylene oxides, acrylonitrile, methacrylonitrile, and methylmethacrylate to starch and dextrin. The influence of composition and structure of the graft copolymers on properties such as solubility, viscosity, and polymer melt temperature were established. Some of the products have properties indicative of utility in one or more industrial applications. However, cost of the products, which will be relatively high owing to the use of anhydrous DMSO as solvent for the reactions, must be determined before their commercial potentialities can be appraised.

5. Thermal reactions of starch. The problem of heat transfer at low pressure in the fluidized bed reactor for converting starch to levoglucosan was successfully solved. The reactor can now be operated in stable equilibrium conditions for unlimited times. An outgrowth of this work is the possibility of activating starch for reaction by utilizing its dielectric properties. A grant to investigate this possibility has been made to the University of Pittsburgh.

In studies under a PL 480 grant to the University of Edinburgh, Edinburgh, Scotland, the thermal stability of starch and its components from 150° to 350° C. was investigated in order to characterize various types of starches and their components. In the presence of small amounts (2 percent) of simple inorganic salts such as sodium chloride and bicarbonate, the threshold temperature of decomposition was significantly lowered and production of volatile products was increased.

6. Starch polyol foams. Studies on rigid urethane foams based on starch polyols showed that stability to accelerated aging (4 weeks at 70° C. and 100 percent relative humidity plus 1 week at 100° in a forced-air oven) equaled that of commercial foams. Addition of a commercial organophosphorus compound to the polyether system imparted fire retardancy to the final foam. Rigid foams containing over 90 percent open cells were obtained by use of α-sulfostearic acid for neutralization of the alkaline catalyst employed in preparing polyols by the reaction of propylene oxide with glycol glycosides. It was also discovered that foams with acceptable stability can be made when up to 35 percent of a commercial gum-type corn starch dextrin is included in the formulation. These foams are self-extinguishing without addition of conventional fire retardants. (Pilot-plant studies on starch polyol foams are reported under subheading 1-D-2.)

7. Chemical products from starch. In experiments with cyanoethylated pearl starch, partial hydrolysis to acid and/or amide groups and limited alkaline hypochlorite oxidation of starch hydroxyls to carbonyl and acid groups gave a series of derivatives with viscosity characteristics suitable for paper-coating applications. Permanganate oxidation of starch eliminated set-back tendencies of pastes but, the oxidized starches were unsuitable as paper-coating adhesives because of poor paste clarity and deposition of sediment on cooking.

Up to 36 percent of the relatively high-priced isolated soy protein could be replaced with the less expensive soy flour if a small amount of dialdehyde starch (DAS) was included in a conventional formulation for pigmented paper coatings. Very good wet-rub resistance was obtained and other coating properties such as brightness and wax pick resistance were essentially unaffected. Exterior exposure studies showed good durability (7 months) of southern pine plywood bonded with protein-DAS glue. All specimens failed in 9 months, whereas commercial resin-bonded plywood remained in good condition for 11 months and showed failure of one-third of the specimens during the 12th month.

Products obtained by crosslinking starch xanthate in water with high-molecular-weight polyethylenimine were incorporated with pulp into paper handsheets. Improvement in wet- and dry-strength was comparable to that achieved with starch xanthide. Retention was 100 percent. Several starch-based products were successfully used as reinforcing agents for natural and synthetic rubbers. More detailed evaluation will be performed by the University of Akron under a research contract.

Contract research on retention of starch xanthates and xanthides by wood pulp was initiated at the Institute of Paper Chemistry. First results revealed that starch xanthate of 0.12 D.S. was not significantly retained on bleached softwood kraft fibers under a wide range of fiber concentrations, pH, alum content, and contact time.

At the Ahmedabad Textile Industry's Research Association, Ahmedabad, India, various hydroxy ethyl glucose derivatives are being prepared for use as reference compounds in studies designed to elucidate the structure of hydroxyethylated corn starch. In other research at this institution, a practical procedure was devised for preparing copolymerization products of starch and locust bean gum and of starch and guar gum. These investigations were conducted under PL 480 grants.

8. High-amylose (HA) starch films and chemical derivatives. A commercially feasible procedure was developed for preparing the triacetate of HA starch. Hydroxypropyl, methyl, and carboxymethyl ethers of HA starch were prepared for evaluation of film properties. Activation of HA starch by pretreatment with dimethyl sulfoxide was the most effective method for achieving rapid acetylation or chlorination with phosphorus pentachloride. The chlorinated starch could be converted to starch amines by reaction with aliphatic amines.

C. Microbiology and Fermentation

1. ARS Culture Collection. As of January 1, 1966, the ARS Culture Collection contained 17,070 permanent cultures. During 1965, a total of 2,228 cultures were distributed to domestic and foreign recipients. Twenty-four samples of reportedly toxic fescue were examined for unusual fungi not common to nontoxic fescue or to orchard grass. Approximately 70 isolates were retained. (See Area 17 of this report.) All new type-cultures (88) of Aspergillus described in the new book on this genus by Raper and Fennell (University of Wisconsin) have been deposited in the culture collection.

Contract research by the American Type Culture Collection revealed that 94 percent of the fungi scheduled for study remained viable after freezing and storage for 6 months in liquid nitrogen. Those that failed this test did survive when 10 percent dimethyl sulfoxide was substituted for 10 percent glycerol as the suspending medium.

Research of importance to the ARS Culture Collection is in progress at several foreign institutions under PL 480 grants. At the National Institute of Agronomic Research, Madrid, Spain, studies have been initiated on the distribution of aerobic actinomycetes. Techniques have been developed for the isolation, characterization, and assay for antibiotic-producing activity of soil isolates. The taxonomic characteristics of 49 soil isolates of actinomycetes, including electron photomicrographs of spores of representative cultures, were submitted to the Northern Division. The cultures themselves were likewise submitted for further study.

Aerobic actinomycetes are being collected by scientists of the Central Drug Research Institute, Lucknow, India, with particular emphasis on strains producing antibiotics active against Agrobacterium tumefaciens, the organism responsible for galls on many economic plants. About one-third of the actinomycete cultures isolated so far inhibited the growth of A. tumefaciens on agar media. These cultures represent various soil types. Initial experiments to produce the antibiotic in liquid fermentation media gave variable results.

At the University of Allahabad, Allahabad, India, additional new species of the order Mucorales were discovered. In other studies at the University of Allahabad, it was established that there is no significant difference in yields of fumaric acid, itaconic acid, penicillin, and amylase from lyophilized and nonlyophilized cultures of the respective fungi producing those materials. There has been no marked reduction in viability of lyophilized cells of selected species of Streptomyces during storage for 1 year.

2. Taxonomic investigations. In taxonomic studies of Mucorales, the zygosporic stage of the species Rhizopus chinensis has been discovered for the first time. This and other small-spored species of the R. chinensis group have now been found in molded peanut meal. During the work, a disease of Rhizopus was observed in which Rhizopus is attacked by species of the genus Syncephalis. Some species are strongly parasitic on R. oligosporus, the tempeh mold. A method for growing isolates of Syncephalis in pure culture was discovered. Electron microscopic studies showed that no Rhizopus strain examined had sporangiospores with smooth walls. This observation casts doubt on the validity of the "smooth-spored" criterion in taxonomy of these microorganisms.

In studies on yeasts, characterization of the protosexuality of Hansenula wabatongushiensis has been completed. Hybridization was found to be the most objective of all criteria for taxonomic speciation of yeasts.

3. Studies on enzymes. Studies made of the relative proportions of the two glucoamylase isozymes produced by Aspergillus niger NRRL 3112 showed that one predominates in the culture for up to about 48 hours. Later, the second becomes predominant and continues to increase for up to 96 hours. After the first 48 hours there seems to be no further increase in the amount of the first isozyme; in fact, drastic decline is occasionally observed. Glucoamylases from different strains of black Aspergilli were found to be similar. A method was developed for automatic assay of glucoamylase.

Research under the grant to the University of Nebraska showed that Rhizopus delemar produces carbohydrase isozymes as does Aspergillus niger. All glucoamylases from A. niger had similar molecular weights (125,000 ± 6%) as determined by ultracentrifugation but isoelectric points were different.

These isozymes also had identical action patterns and N-terminal amino acid residues (L-alanine) and were apparently stable to reagents that promote dissociation into subunits.

Research on coupled enzyme systems was initiated with studies on formation and properties of a glucose isomerase produced by Aerobacter aerogenes and a stable D-glucose 6-phosphate dehydrogenase produced by Neisseria perflava. The glucose isomerase appears to be phosphohexoisomerase. The glucose-6-phosphate dehydrogenase was substantially purified but separation from myokinase activity was not achieved. At all stages of purification, relationship of dehydrogenase activity to nicotinamide-adenine dinucleotide (NAD) or to nicotinamide-adenine dinucleotide monophosphate (NADP) as coenzymes remained constant. This enzyme, which is the first NAD/NADP-dependent glucose-6-phosphate dehydrogenase discovered in a gram negative organism, possesses remarkable stability that should greatly simplify its evaluation for converting cereal grains to industrial materials.

In other studies to find enzymes that might be useful for chemical conversions, a mutant strain of Candida tropicalis Y-1367 was found capable of producing 9,000 units/ml. of glucosyl transferase. This enzyme, which converts glycerol in solutions containing dextrin to glycerol glucoside, was purified 100-fold, making possible more accurate determination of several of its characteristics. Several promising strains of organisms capable of producing D-glucarate dehydratase have been isolated from soil.

Initial studies on spores as agents for effecting chemical conversions involved a survey of 20 species of molds. Spores from all species showed metabolic activity on most of the substrates tested, including fatty acids, other organic acids, amino acids, hydrocarbons, and terpenoids. With each of several preparations tested, spore activity increased with age. These results are encouraging not only because activity has been demonstrated but also because a wide variety of substrates can be metabolized by spores.

Under a grant to Iowa State University, action patterns of amylases from a number of microorganisms are being determined. Amylase from Thermoactinomyces vulgaris converted amylose primarily to maltotetraose. That from Streptococcus bovis converted starch first to maltotetraose and maltose; later, significant amounts of maltotriose and glucose were formed.

At Kansas State University, also under a grant, a new method for staining acrylamide gels was developed that will accelerate analysis of enzymes obtained by preparative gel electrophoresis.

The program of research on enzymes has been broadened by a grant to the University of Arkansas for basic studies on the mechanism of enzymatic hydrolysis of cereal starches. Research under this grant has not yet been initiated.

Lytic enzymes of microbial origin are being studied under a PL 480 grant to the University of Liege, Liege, Belgium. Three distinct lytic endopeptidases from the Streptomyces albus G enzyme complex have been isolated and characterized. They are highly active on lysine-containing cell walls of gram positive bacteria. By use of these specific endopeptidases in conjunction with other enzymes from the Streptomyces complex, structures of four different types of peptide bridges linking peptidoglycan units of cell walls were elucidated.

Work on the mechanism of action of the F_1 β-1,4 endo-N-acetylmuramidase from Streptomyces showed that (1) pH optimum for lytic activity depends on the structure and composition of the bacterial wall, (2) affinity of the F_1 enzyme for N-acetylmuramyl linkages is greatly enhanced by peptide substitution of these residues, (3) few walls from gram positive bacteria possess the tight network peptidoglycans found in Staphylococcus aureus and Micrococcus roseus.

4. Biological insecticides for Japanese beetle. Refractile spores of Bacillus popilliae have been repeatedly produced in liquid media containing 1 percent of activated carbon. Yields range from 10,000 to 100,000 per ml. These spores have withstood drying in sterile soil and storage for 6 months. They have also survived for at least 4 months in sterile sand and in a $CaCO_3$-talc mixture like that used in commercial spore preparations. The most consistent sporulation in shaken cultures was achieved with activated carbon made from domestic nut shells; different activated carbons differed greatly in effectiveness.

A new, infective derivative strain, B-2309M, sporulates consistently on agar medium. About 10-20 percent of the cells on a plate form spores.

Improvements in media have resulted in more vigorous and reproducible growth of B. popilliae. Populations of 1.6 billion viable cells per ml. are now regularly attained in 18-20 hours.

Study of sporogenesis of B. popilliae in larvae showed exclusive and limited vegetative development in the hemolymph for the first 4 or 5 days. At this time, pre-spore forms abruptly appear and predominate, followed by sporulation to about 80 percent by the ninth day. Sporulation finally reaches 90-95 percent by subsequent slow accumulation from vegetative cells remaining after the initial sporulation.

Investigation of the proteins of grub hemolymph revealed a high-molecular-weight major fraction plus small amounts of four other protein fractions of lower molecular weight. The latter increase in concentration at the expense of the high-molecular-weight fraction during the course of milky disease. Also, the oxygen level in hemolymph was found to increase during sporulation of vegetative cells.

Serological studies based on successful preparation of rabbit antisera to surface antigens of vegetative cells and spores of B. popilliae and B. lentimorbus have been undertaken. The most significant of the initial results are that both spores and vegetative cells possess a common antigen; that antigen content changes with cell age; and that B. popilliae vegetative cells are not antigens for spore antiserum. These results may provide a way to detect the point at which commitment to sporulation occurs.

In the contract research at Baylor University, electron microscopy of thin sections of cells of B. popilliae in different stages of sporulation, together with phase microscopy of sporulation on solid media, is providing a highly detailed picture of the entire process and of the intimate structural changes that transpire. Spores prepared by the NU plate culture procedure were shown to be true spores. Significant structural differences between vegetative cells from infected larvae (pre-symptom stage) and those grown in the laboratory were observed.

Study of enzymes in sporulation under the contract at Michigan State University involved a comparison of enzymes of spore-like bodies produced by B. popilliae and B. lentimorbus cultured on media containing barbiturate or β-hydroxybutyrate (BHB). Extracts of spores produced in vivo and of spore-like bodies produced in vitro had similar enzyme activity. Activity was found generally to be influenced by BHB, although changes in activity for corresponding enzymes from the two parent microorganisms were not parallel. As much as 80-90 percent "sporulation" of B. popilliae was obtained in a liquid medium containing BHB. The "spores", however, could not be germinated. Barbituric acid was found to specifically influence transition of vegetative cells in liquid media to spore-like bodies.

Contract research at Kansas State University has resulted in a lyophilization procedure that achieves survival of up to 83 percent of vegetative cells of B. popilliae.

5. Microbial polysaccharides. Engineering research on continuous fermentation with B-1459 showed that polymer formation is not associated with cell growth but takes place during the entire fermentation at an essentially constant rate. A pilot-plant two-stage continuous fermentation was operated for 5 days with an overall yield of 62 percent based on glucose added. Microbial contamination was present, but did not appear to affect the viscosity of the product in comparison to uncontaminated continuous runs. In a 6-day run, modified by intermittent addition of inoculum, polymer yield was about 60 percent. In this experiment, microbial contamination occurred at 2 days but did not influence the fermentation until the fifth day. Based on kinetic studies on the growth of the B-1459 organism, an improved batch fermentation procedure was developed that reduces fermentation time from 96 to 48 hours.

Under the grant to Cornell University, electro-deposition was used successfully for removal of polysaccharide B-1459 from fermentation broths. Rates

of 0.5 mg/cm^2/hr could be obtained. It was also observed that resting cells produced polysaccharide in the same yield as growing cultures. Toxicity of hydrocarbons below C_{11} for use in emulsion-type fermentations was confirmed.

Studies on the structure and chemistry of polysaccharides showed that the β-D-mannuronic acid linkage, found in B-1973 and certain other polysaccharides, possesses inherent instability that can lead to anomalous chemical behavior. Although the mode of linkage of pyruvic acid appears to be the same in polysaccharides B-1828 and B-1459, the percentage of this acid in B-1828 is more consistent among various preparations than in B-1459 and the stability to acid hydrolysis is much greater. Structure studies on Y-6493 and Y-6502 phosphogalactans have been completed. Results show that phosphate occurs exclusively as α-D-galactosyl-1-phospho-6'-galactosyl end groups. The remaining galactosyl linkages are α-1,3 and α-1,6 in about equal proportions. A chemical-chromatographic technique has been developed that simplifies identification and quantitation of complex mixtures of monosaccharides obtained by acid hydrolysis of polysaccharides. The aldoses are reduced to alditols, fully acetylated and separated by gas-liquid chromatography.

The extracellular polymer produced by Y-6272 has been separated into three components: a galactomannan, a glucomannan, and an N-acetyl glucosamine polymer that is combined or complexed with amino acids. Optimal conditions were established for production of this polymer.

Structure studies were strengthened by a grant to the University of Wisconsin for studies on the fine structure of polysaccharide B-1459.

It is timely to note that the Northern Division's research on microbial polysaccharides appears to have founded a new type of industry. Evidence has been accumulating which shows that industrial activity has transcended interest merely in the Northern Division's specific polysaccharides. Stimulated by our research and development program in this area and, undoubtedly, by the favorable acceptance of B-1459 as a commercial product, industry has gone on to independent research and promotion of independently discovered products. For example, one company is actively investigating derivatives and modifications of B-1459. Several companies are screening new microbial sources. Two others are actively seeking markets for products which they themselves discovered and developed. If this trend continues, microbial polymers may join antibiotics as industries opened up by the Northern Division's research.

6. Plant antibiotics. Preliminary results of tests conducted by the Boyce Thompson Institute on plant antibiotic preparations furnished by the Northern Division showed that several were effective against one or more of 12 economically important fungal diseases of plants. One (F-17) inhibited six diseases, but none was active against either Dutch elm disease or nematode infestation.

7. Microbiological processes and products. In studies on genetic control of fermentation, a phenomenon of spontaneous reversion from the mutant state to "wild-type" at high incidence upon dilution of cultures was discovered. Unrecognized, this new phenomenon appears to have misled previous investigators who had reported conjugation in Pseudomonas aeruginosa. The phenomenon was found using the mating-type strains and the system on which reports of conjugation in the literature were based, as well as with new mutant strains prepared at the Northern Division. It is concluded that conjugation in P. aeruginosa has not been established. However, the pronounced effect of dilution on reversion is unique and may offer a clue to the genetic processes of pseudomonads.

The strain of Hansenula holstii NRRL Y-2448 used for the production of phosphomannan is diploid. By appropriate techniques, haploid strains now have been derived from it which produce either little or much phosphomannan. When these haploids are judiciously crossed, new diploid cultures are obtained which produce phosphomannans which differ qualitatively and quantitatively from that of the parent cultures. Results of genetic analysis suggest that there are several lethal factors which affect spore formation and germination. Selective breeding experiments are being done to overcome this restriction in spore viability without changing the inherent capacity of the organism for polymer production. If successful, more complete analysis of factors affecting phosphomannan formation can be done and perhaps modifications of phosphomannan capacity can be introduced by breeding and by mutation.

In the Pioneering Laboratory for Microbiological Chemistry, investigation of a genetic defect of Rhodospirillum rubrum that interferes with identification of mutants on the basis of colonial morphology revealed that the lipopolysaccharide content of cell walls may be implicated in this phenomenon. Preliminary studies of an ornithine-containing lipid present in large quantities in R. rubrum showed that it has no free carboxyl and only one free amino group. Although it behaves chromatographically like a phospholipid, it does not contain phosphorus. Experiments with radioactive tracers suggest that this lipid is related to the arginine biosynthetic pathway. Further investigation of macromolecules responsible for agglutination of certain yeast strains confirmed that disulfide bonds have a specific function in the sex-specific agglutinin and may, in fact, be involved in the agglutinative site.

In other work in the Pioneering Laboratory, a new acid of m.p. 130° was isolated from the extracellular lipid produced by the yeast YB-2501, the taxonomic position of which has not yet been established. The acid was shown to be 13-keto-8,9-dihydroxydocosanoic acid by periodic acid cleavage to two aldehydes which on oxidation yield suberic acid and 5-ketotetradecanoic acid. The configuration of the 8,9-hydroxyls was shown to be erythro by conversion of erythro-8,9,13-trihydroxydocosanoic acid of m.p. 156° to the new acid via oxidation of the 13-hydroxyl group after acetonation of the 8,9-hydroxyls. A detailed study of the hydrogen

bonding system in ramulosin is in progress. Eleven halogen derivatives have been prepared and subjected to ultraviolet, infrared, and NMR analysis. Infrared and NMR examination of ramulosin itself indicated that it exists partly as the 6-membered hydrogen chelate and partly as a 12-membered dimer.

In studies under a PL 480 grant at the University of Milan, Milan, Italy, the levels of 2-ketogenic and of 5-ketogenic activities in Acetobacter suboxydans were found to be correlated to particular vegetative states, rather than directly determined by external inducers such as substrates (glucose, gluconate, sorbitol, mannose, glycerol), amino acids, or vitamins. It was concluded that these enzymatic activities are constitutive in the organism. Therefore, it does not appear that fermentation conditions can be established by which only 5-ketogluconic acid will be produced. This research has been completed.

At the University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England, scientists have purified the teichoic acid of Streptomyces griseus and shown it to be a mixed polyphosphodiester of glycerol and ribitol. The gram positive bacteria, in contrast, contain separate polyglycerol and polyribitol phosphates with alanine ester and glycosyl groups linked to the polyols. The S. griseus teichoic acid contains β-glucopyranosyl substituents on some of the ribitol units and small amounts of acetic and succinic monoesters in place of alanine. Alkaline hydrolysis and phosphomonoesterase treatment gave rise to two new phosphate esters: ribitol 3-phosphate, which is resistant to hydrolysis by alkaline phosphomonoesterase; and ribitol 3-phospho-1'(3')-glycerol. This research is being conducted under a PL 480 grant.

8. Fermentation acids. Research on fermentation acids is in progress under PL 480 grants to several foreign institutions.

At the University of Lodz, Lodz, Poland, mutation of Aspergillus terreus by radiant energy yielded several strains of the organism with improved ability to produce itaconic acid. Yields of itatartaric acid per se in the fermentation are still rather low, but this may be due to instability of the acid. Indoleacetic acid was found to stimulate both itaconic and itatartaric acid.

In research at the University of Tokyo, Tokyo, Japan, approximately 1,000 cultures of yeasts, molds, streptomycetes, and bacteria were screened for production of mevalonic acid. Nearly all of the organisms formed some mevalonic acid; however, only members of the genera Aspergillus, Hansenula, Pichia, and Endomycopsis produced significant quantities. Although no organism produced enough mevalonic acid to provide a basis for economic production, the results do indicate that continued survey, improved cultural conditions, and use of mutant strains may eventually lead to a commercially useful process.

In other research at the University of Tokyo, many strains of bacteria, yeasts, and molds have been screened for their ability to produce tartaric acid. Only two strains of bacteria showed possible production of tartaric acid in small quantities. There is now some question whether or not the acid produced by these organisms is really tartaric acid. If not, a new approach to the problem will undoubtedly be needed.

D. Technology - Process and Product Development

1. Cereal xanthides and xanthates. Studies on stability of the xanthide group in solution showed that when treated with base, model compounds (glycol derivatives) containing a xanthide group and a free hydroxyl group underwent fragmentation to yield thionocarbonates (1,2- and 1,3-structures) or S-alkyl xanthate esters (1,4-, 1,5-, and 1,6-structures). The 1,2-glycol monoxanthide yielded the thionocarbonate on standing without treatment. These studies were extended to amylose xanthate and to an amylose-derived model compound blocked in the 6 position. Both compounds rearranged in a manner similar to that of simpler models to yield the thionocarbonates. However, with amylose xanthate itself, which contained no blocking group, the ring of the initially formed thionocarbonate opened with further reaction to yield intermolecular, acyclic thionocarbonates. A byproduct of these studies was development of a facile method for preparing cyclic thionocarbonates that are valuable intermediates for introduction of unsaturation into carbohydrates. In other studies on stability, it was observed that treatment of a precipitated xanthide with dilute acid or a cation exchange resin followed by drying at 80° C. gave products that did not change during storage for several weeks. When starch xanthide (D.S. 0.12) was suspended in water and drum dried at 110-115° C. for 2 minutes, there was no loss of sulfur. The dried products contained 6-8 percent moisture and did not lose sulfur during storage for 4 months at room temperature.

A continuous process has been developed in which starch xanthate is crosslinked *ex situ* with chlorine gas and immediately metered into wood pulp flow lines. Results with this process were superior to those obtained with the continuous *in situ* process described in last year's report.

In cooperation with Forest Products Laboratory, 500 lbs. of unbleached southern sulfate pulp was refined according to the Northern Division's specifications. This pulp, which was typical of linerboard stock, was used in evaluation of the continuous process for incorporating xanthide. Results indicating definite potential for promoting crush resistance were obtained in tests at 90 percent R.H. of linerboards containing starch xanthides crosslinked by all of the principal processes developed at the Northern Division. Xanthated commercial corn flour was nearly equal to starch xanthate in improving linerboard.

A large pilot-scale papermaking machine and related equipment have been installed at the Northern Division. With this facility xanthates, xanthides, and other products made from cereal starches and flours can be evaluated under conditions closely resembling those existing in commercial plants of the paper industry.

Contract research at Battelle Memorial Institute on pilot-plant production of xanthides has been completed. Final phases of the work showed equal effectiveness and perhaps a slight cost advantage for chlorine as compared to hypochlorite for crosslinking. Hydrogen peroxide had no functional or economic advantages over chlorine and hypochlorite. Several methods of preparing ex situ crosslinked xanthides were devised. Although the ex situ preparations displayed activity as wet-strength agents in paper equal to that of in situ preparations, they are too expensive and have inadequate shelf life to be commercially acceptable.

2. Starch polyol foams. Contract research on scaling up the starch polyol process, preparing rigid urethane foams with continuous foam-making machines, and evaluating the products has been successfully completed by the Archer Daniels Midland Company. Batches as large as 1,000 pounds were prepared. The foams had good dimensional stability and flame resistance. Their properties equaled those of foams based on methyl glucoside and were superior to those of foams based on sorbitol. Preliminary design and plant investment and operating cost calculations were completed by Archer Daniels Midland Company. Plant gate cost of the polyol for a plant producing 10,000,000 pounds per year was estimated at about 15 cents per pound. (See also subheading 1-B-6.)

3. Graft copolymers. Under a research contract, two 100-pound lots of starch-acrylonitrile graft copolymers were prepared at Stanford Research Institute in its continuous reactor. Irradiation with gamma-rays was found to be less efficient than irradiation with an electron beam in the large-scale preparation of starch-acrylamide graft copolymers. Construction of a pilot plant for continuous production of this copolymer by electron beam irradiation was completed. On the basis of a study of the effects of reaction variables, two 100-pound lots of each of two different starch-acrylamide copolymers were prepared in this pilot plant. (See also subheading 1-B-4.)

4. Starch derivatives for protective coatings. Contract research at Battelle Memorial Institute has shown that four starch derivatives--cyanoethylated corn starch, potassium carboxymethyl starch, a commercial cationic starch, and a commercial hydroxyethylated starch--have promising properties as viscosity control agents in emulsion paints. Extensive evaluation is planned.

Research to find outlets for starch derivatives in the protective coatings industry was strengthened by a contract with Archer Daniels Midland Company.

This contract provides for an investigation of starch-derived glycol and glycerol glycosides in alkyd resins and other coating compositions.

5. Cyanoethylated starch. Large samples of cyanoethylated starch (200 pounds) and cyanoethylated acid-modified starch (450 pounds) were prepared in the pilot plant for use in contract research at Western Michigan University. A simple laboratory process for cyanoethylating starch was successfully devised. In the process, a cake prepared from starch wetted with dilute NaOH solution and dried to 18 percent moisture is allowed to stand in an atmosphere of acrylonitrile vapor. A D.S. of 0.07 was achieved in 50 hours.

At Western Michigan University all experiments in coating and sizing paper with cyanoethylated corn starches have been completed. Samples of all products have been received at the Northern Division, but the contractor has not yet forwarded test results.

6. Corn dry milling. Investigations on dry milling of corn are directed to development of improved products for food and industrial use. Results of this work are reported under Area 2, subheading C-1.

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Technology - Process and Product Development

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AREA NO. 2: CORN UTILIZATION - FOOD

Problem. Utilization of corn in products for human consumption is an outlet of great economic importance. In 1964, U. S. per capita consumption of meal, cereal food, sirup, sugar, and starch obtained from corn totaled 28.3 pounds. If corn used for production of alcoholic beverages is included, this figure would be about 25 percent greater. In processing corn for food and beverage uses, corn oil is obtained as a valuable byproduct. Since 1964, annual production of corn oil has exceeded 400 million pounds. Except for foots and refining losses, all of this oil is consumed in food products, principally margarine and salad and cooking oils.

The need for research is encountered primarily in dry milling of corn where the yield and quality of fractions are important both economically and in terms of consumer acceptance and nutritive value of final products. Improvements are needed in milling techniques, especially for old and artificially dried corn, if optimum results are to be obtained. More information is needed on the composition of corn and corn fractions in order to identify and minimize losses of nutritionally important components that may occur during processing. Such investigations should result in cheaper and more nutritious products and therefore contribute to increased utilization.

In addition, these studies provide a foundation for research on composition, processing, and utilization of new strains of corn now being developed that have significantly higher nutritional value than does ordinary corn. Success in this development could make corn the world's No. 1 food grain. U. S. corn production, which is now 4 billion bushels annually, could be readily expanded to neutralize the present 1-billion-bushel annual world shortage of cereal foods. Since this new corn differs physically from ordinary corn, much effort will be needed to develop suitable milling methods and to provide the knowledge necessary for development of suitable food products to meet dietary needs of world populations.

It has recently been discovered that certain oilseeds and cereal grains, including corn, are subject to infection by molds that can produce toxic products. To provide safe food products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from corn.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of corn in food.

The Federal scientific effort for research on food utilization of corn totals 4.8 scientist man-years. Of this number, 1.2 are devoted to chemical composition and physical properties; 2.2 to microbiology and toxicology; and 1.4 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (1.2 scientist man-years) is concerned with lipids and proteins of the corn kernel. During the reporting period, studies on nonprotein nitrogen compounds of corn were completed.

Research at Peoria, Illinois, on microbiology and toxicology (2.0 scientist man-years) is devoted to studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.2 scientist man-year) is allocated to research on food uses of corn.

Research conducted at Peoria, Illinois, on technology - process and product development (1.1 scientist man-years) comprises investigations on corn dry milling. A grant has been made to Pennsylvania State University, University Park, Pennsylvania, for basic studies on the mechanical and viscoelastic properties of shelled corn as related to the corn dry-milling process (.3 scientist man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 6.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Corn proteins, lipids and nonprotein nitrogen components. Compositional studies on corn are relevant both to food and feed utilization. Results are reported under Area 3, subheading A.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are relevant both to food and feed utilization of corn. Results are reported under Area 3, subheading B-2.

C. Technology - Process and Product Development

1. Corn dry milling. Studies on corn dry milling showed that stress-crack formation increased as the initial moisture content of the corn decreased.

Stress-crack formation also was influenced by the temperature of tempering and took place most rapidly in the 65-85° F. range. Pretempering increased the yield of -4+6 grits by about 50 percent for two lots of corn dried under severest "dryeration" conditions. Improved results were obtained when the cold-tempering procedure was modified by adding the temper water in five rather than two steps. Results did not, however, equal those of pretempering or tempering with a steam-water mixture. Water flotation was found to be a promising means for removal of germ and some grit with attached germ from corn degerminator product in the $-3\frac{1}{2}+25$ mesh range.

2. Mechanical and viscoelastic properties of corn kernels. Research in progress under the grant to Pennsylvania State University showed that swelling stresses occurring in the corn kernel during moisture absorption can be interpreted by treating the kernel as a cellulosic gel. A number of methods and techniques developed for other gel structures, such as wood, presumably should be applicable to corn.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

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Color, Texture and Other Quality Factors

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*Research supported by PL 480 funds.

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Technology - Process and Product Development

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AREA NO. 3: CORN UTILIZATION - FEED

Problem. Over 90 percent of the U. S. annual production of corn is used as animal feed. Corn is fed to animals in various forms including ear corn, shelled corn, cracked or ground corn and, in certain mixed feeds, corn gluten and other milling fractions. Because of the extremely large volume of this outlet, even small improvements in quality or processing efficiency are economically important to the feed industry and to the farmer.

The components responsible for certain nutritional effects attributed to corn, such as growth stimulation and improved feed utilization efficiency, have not been satisfactorily identified, nor are processing steps available that take these components into account. More information is needed generally on the nutritionally important components of corn and on the changes that occur in them during processing. Besides their activity as Vitamin A precursors, carotenes contribute desirable color to milk and the body fat of cattle. Xanthophyll pigments similarly impart yellow color to egg yolks and to the skin of broilers and fryers. Adequate information on these pigments and on their fate during processing is also needed to insure maintenance and improvement of quality.

Compositional and related processing research is required on several new strains of corn that are expected to become commercially important. These strains include (1) those expected to lead to corn capable of providing a balanced source of amino acids, and (2) those that contain increased amounts of xanthophyll and other carotenoid pigments and therefore would improve the competitive position of U. S. corn in world markets.

Another important direction for research is the fermentative conversion of corn grain and corn sugar to nutritionally important feed additives. The value of corn-based media for production of vitamins, β-carotene, and antibiotics is well known. However, possibilities for additional important developments are virtually unlimited and should be investigated on a continuing basis. Corn steep liquor is frequently used as a supplement in fermentation media and may also be added to feedstuffs. More information is needed on corn steep liquor to identify the factors responsible for its stimulating effects on growth.

It has recently been discovered that certain oilseeds and cereal grains, including corn, are subject to infection by molds that can produce toxic products. To provide safe feed products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from corn.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of corn in feed.

The Federal scientific effort for research on utilization of corn in feeds totals 3.1 scientist man-years, of which 1.1 are devoted to chemical composition and physical properties and 3.3 to microbiology and toxicology.

Research at Peoria, Illinois, on chemical composition and physical properties (1.1 scientist man-years) involves study of lipids and proteins of corn kernels. During the year, research on corn carotenoid pigments and on nonprotein nitrogen compounds was completed.

Research at Peoria, Illinois, on microbiology and toxicology (2.0 scientist man-years) is concerned with studies on the production of mycotoxins by Aspergillus flavus and related molds. Research contracts (1.3 scientist man-years*) are in effect with A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene; with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene; and with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains. Effort on this project is prorated among corn, wheat, and sorghum. During the reporting period, research was completed at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, on production of Vitamin B_{13} and at the National Institute for Agronomic Research, Paris, France, on mutation of yeasts for improved feeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nonprotein nitrogen components. In addition to previously reported nucleotides, the following compounds from the whole mature kernel and endosperm were characterized: nicotinamide adenine dinucleotide, thiamine

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*Work covers more than one commodity; only effort allocated to corn is included in total.

and uridine monophosphates, and guanine diphosphate. Two nucleotides containing thymine were found exclusively in the endosperm. Yields of nucleotides (weight basis) declined during maturation of corn. Larger contents of adenine and uridine nucleotides were found in immature corn, whereas cytosine and thymine nucleotides appeared only in the later stages of development. These studies, now completed, have been replaced by an investigation of the distribution, composition, structure, and properties of proteins from corn, including different genetic strains.

2. Carotenoid pigments of corn. Carotenoid pigments were determined in 575 corn samples furnished by Crops Research, but xanthophyll levels did not exceed the high value of 77 p.p.m. given in last year's report. These results confirm that breeding can raise the xanthophyll content of corn above the value of 60 p.p.m., a level at which 60 percent corn in poultry feed will provide a sufficient source of pigment for eggs. This research has been terminated.

3. Lipids in hybrid corn kernels. Preliminary studies have confirmed a satisfactory method for extracting "free" lipids from ground yellow corn and corn fractions. A thin-layer chromatographic technique appears to give adequate separation of the lipids into five classes: (1) unknown, probably includes phospholipids; (2) mono- and diglycerides or sterols; (3) free fatty acids; (4) triglycerides; and (5) hydrocarbons.

B. Microbiology and Toxicology

1. Microbial carotenoids. Studies on factors influencing biogenesis of xanthophylls in Chlorella variegatus provided evidence that these pigments are an integral part of the electron transport system. The concentration per cell cannot, therefore, be significantly changed. This research has been terminated.

Contract research by the A. D. Little Company showed that good stability could be achieved by extrusion of crude fermentation β-carotene with carriers such as gum arabic, carboxymethyl cellulose, and mixtures of dextrose with gelatin or starch. Reported stabilities included values as low as 77 percent after 5 months and as high as 100 percent after 7 months. At Consolidated Laboratories, Inc., a number of mutant strains of the β-carotene organism were isolated, but none gave higher yields than strains already available.

2. Aflatoxin investigations. In engineering studies all commitments for quantities of aflatoxin, either as whole metabolite or as a dry crude extract, have been met. The process used for production gave yields of aflatoxin B_1 exceeding 1 g./kg. of solid rice substrate. An extraction procedure was devised that gave a dry powder containing 70 percent of aflatoxin B_1. Products have been provided to the Western Division, to the Food and Drug Administration, and to the National Animal Disease Laboratory.

Strains of Aspergillus flavus were found that produce mainly aflatoxin G_1 on wheat and soybeans as substrates (ratio B_1:G_1 = 1:2). Aflatoxin was produced by three A. flavus strains when grown on oat hulls or groats. Production of aflatoxins at 11° C. by A. flavus was demonstrated.

Degradation of aflatoxin by 11 Pseudomonas spp. was found to be nonspecific. However, both growing and resting cells of Flavobacterium aurantiacum removed aflatoxin irreversibly from several test systems. Milk, corn oil, peanut butter, peanuts, and corn, artificially contaminated with aflatoxin for test purposes, were detoxified by addition of F. aurantiacum cells. Tests with ducklings showed that removal of aflatoxin by viable bacterial cells was complete and no new toxic products were formed. Contaminated soybeans were partially detoxified. Autoclaved cells and cell walls took up aflatoxin, but it could be eluted by repeated washing.

Forage crops (alfalfa, timothy, mixed red clover, sweet clover, and oat straw) were investigated as substrates for aflatoxin production. Toxic levels were produced on timothy, sweet clover, and oat straw by two strains of A. flavus. Aflatoxins B_1 and G_1 were found in all samples of spores of A. flavus NRRL 2999. Aflatoxin was found to reduce early growth of seeds (11 plant species) germinated in its presence.

Aflatoxin analysis was greatly expedited by development of an automated procedure for reading thin-layer chromatographic plates.

In the survey of commercial grains for incidence of aflatoxin, 1,054 samples of corn had been assayed as of June 1, 1966, with positive results (19 p.p.b. or less) for 34 samples. Of these, 24 were in Sample Grade, 5 in Grade 5, 2 in Grade 4, 1 in Grade 3, and 2 in Grade 2. For soybeans, 6 of 711 samples assayed gave positive results; 4 were in Sample Grade and 2 in Grade 4. One sample of soybeans gave an unequivocal positive result by chemical analysis. The other five contained small amounts (3-6 p.p.b.) of fluorescent factors that interfered with analysis. A combined extract from these five samples was toxic in biological tests with ducklings. Survey of corn and soybean samples is continuing.

Examination of wheat and sorghum samples has been completed. Of 534 samples of wheat assayed, 2 gave positive results; both were in Sample Grade. For sorghum, 6 of 533 samples assayed gave positive results; 4 were in Sample Grade and 2 in Grade 4.

About one-third of 175 samples of oats contained fluorescing factors that interfered with chemical analysis. Biological tests indicated absence of toxic material. An improved procedure which eliminated interfering substances has been devised and will be used in a new survey of oat samples.

Although conclusions should not be drawn until the entire survey has been completed, results so far are reassuring in that almost all of the positive responses were observed in the lowest grades of grain and no extremely high levels of aflatoxin were encountered.

Aflatoxin research has been augmented by a research contract with the University of South Dakota for survey of toxin formation by species of the genus Aspergillus.

3. Vitamin B_{13}. At the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, research has been completed under a PL 480 grant for studies on Vitamin B_{13}. Results showed that this growth factor is not a single compound as some believed. Instead, it is composed of a multiplicity of carboxylic acids, phenols, ketones, and cyclic peptides. Concentration of the growth activity is in the peptidic fraction. The individual cyclopeptides were separated, but their structures remain unknown.

4. Improved feeds by mutation of yeasts. Owing to resignation of key personnel, the National Institute of Agronomic Research, Paris, France, terminated prior to completion its research under a PL 480 grant for investigations of mutant yeasts capable of producing high yields of sulfur-containing amino acids. About 1,800 strains of yeasts will eventually be sent to the Northern Division.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

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AREA NO. 4: WHEAT UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Although the principal use of wheat is as food, a total of about 200 million pounds of wheat starch and flour was consumed by industry in 1963. Loss of this market would detract from the economic value of wheat as a crop. As a food grain, wheat commands a price that is generally unfavorable to its utilization as an industrial raw material. However, in certain areas, notably the Pacific Northwest where corn is not grown, wheat is cheaper than either corn or sorghum. Furthermore, new high-yielding strains of wheat especially suited for this area are being developed. There are many paper mills in this area, and the need for technology to use starch and flour produced there has materialized. Much of the starch now used comes from imported tapioca.

Other possibilities for economic and noncompetitive industrial outlets for wheat are based on use of whole ground wheat and millfeeds, including wheat bran, and on exploitation of the properties of wheat gluten protein, which has unique properties not possessed by other cereal proteins.

Not only paper and paper products, but also coatings, adhesives, thickeners, and plastics offer excellent opportunities for industrial products derived from wheat. A more detailed discussion of industrial outlets for cereal starches and flours is given in Area No. 1, Corn Utilization - Industrial Products.

To achieve the objective, research is needed to learn how wheat flour, starch, and milling fractions can best be modified to provide new and improved properties such as water resistance, dispersibility, paste viscosity, tack, and adhesive bond strength. The possibilities of achieving some of these improvements by modification of the gluten component of flour should be investigated. Conditions must be established for optimum use of industrially promising products now under study such as acid- and enzyme-modified flours and xanthated bran and millfeeds. Basic research should provide leads to other products and processes for future development.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of wheat, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of wheat totals 54.8 scientist man-years. Of this number, 9.2 are devoted to

chemical composition, physical properties and structure; 19.1 to chemical and physical investigations to improve products; 13.8 to microbiology and fermentation; and 12.7 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (7.6 scientist man-years) involves study of wheat flour, starch, and the component proteins of wheat gluten. Research on wheat starch is integrated with that on corn and sorghum starches. The work on wheat includes study of the microscopic and ultrastructure of wheat grains and flours and of changes induced by various treatments. A contract (.6 scientist man-year) is in effect with Purdue Research Foundation for studies on alkaline desulfurization of wheat gluten proteins. Grants (1.0 scientist man-year) have been made to Marquette University, Milwaukee, Wisconsin, for basic studies on intermediates involved in forming glycoprotein linkages; to Iowa State University, Ames, Iowa, for basic research* on heat, mass, and momentum transport of cereal starches and flours; and to Purdue Research Foundation, Lafayette, Indiana, for research* on the effects of disulfide bond cleavage on the structure of corn and wheat endosperm proteins.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (14.0 scientist man-years) includes study of the chemical reactions of wheat starch, flour, protein and milling fractions with the objective of discovering new chemical products and processes having potential for industrial use. Research on wheat starch is integrated with that on corn starch. During the year, one phase of this work involving study of possible means for preparing amino acid derivatives of starch was completed and replaced by research on synthesis of halogen derivatives of starch. Also, a project on hydrophilic derivatives of wheat flour was completed. Acid-modified flour, one of the products developed under this project, is being further evaluated under a research contract. Research contracts (2.8 scientist man-years) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies* on the reaction of acetylene with methyl glucoside; with The Johns Hopkins University, Baltimore, Maryland, for basic research* on the reactions of starch in fluid dynamic media; to the University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; with Southern Illinois University, Carbondale, Illinois, for investigations* on synthesis of maltooligosaccharides; with Stanford Research Institute, Menlo Park, California, for research* on graft copolymers of cereal starches with vinyl-type monomers; with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation* of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper; and with IIT Research Institute, Chicago, Illinois, on preparation, characterization, and chemical modification of polypeptides

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*Work covers more than one commodity; only effort allocated to wheat is included in total.

derived from wheat gluten. Contract research was completed by Ohio State University, Columbus, Ohio, on synthesis of amino derivatives of starch and by the University of Arizona Agricultural Experiment Station, Tucson, Arizona, on the reaction of starch with mercaptans. Grants (2.3 scientist man-years*) have been made to Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; to Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; to Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur; and to the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides.

Research on microbiology and fermentation conducted at Peoria, Illinois (11.7 scientist man-years), includes studies on the use of microorganisms to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. This research is integrated with similar studies based on corn. A large collection of pure cultures of industrially and agriculturally important microorganisms is maintained. The Pioneering Laboratory for Microbiological Chemistry conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (.7 scientist man-year*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation; and at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective. Contract research at the University of Illinois, Urbana, Illinois, on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens has been completed. Grants (1.4 scientist man-years*) have been made to Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes; to Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; to Iowa State University, Ames, Iowa, for investigation on bacterial amylases and their action

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*Work covers more than one commodity; only effort allocated to wheat is included in total.

patterns; to the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; and to the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch.

Research conducted at Peoria, Illinois, on technology - process and product development (5.8 scientist man-years) is concerned with detailed study and evaluation of wheat-derived products having definite potential for industrial utilization and of processes for making them. Also, studies are conducted on modified techniques for milling and fractionating wheat to obtain improved materials for industrial and other purposes. Research involving chemical modification of wheat starch is integrated with that on corn starch. Research contracts (6.9 scientist man-years) are in effect with Stanford Research Institute, Menlo Park, California, for process development* of selected starch graft copolymers; with Battelle Memorial Institute, Columbus, Ohio, for developmental research* on starch and other cereal grain xanthides, for development of optimal processes for incorporating wheat-derived xanthides into paper products, and for studies* on starch derivatives for use as colloids in water-emulsion paints; with Western Michigan University, Kalamazoo, Michigan, for evaluation* of modified cyanoethylated starches for applications in paper; with Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations* on the use of starch glycosides in coatings and plastics; with the Brown Company, Berlin, New Hampshire, for evaluating acid-modified flour as a paper size; and with the University of Akron, Akron, Ohio, for evaluation* of starch and starch derivatives as reinforcing agents for natural and synthetic rubber. During the year, contract research on evaluation of starch polyol urethane foams was completed by Archer Daniels Midland Company, Minneapolis, Minnesota, and studies on pneumatic fluidization of wheat flour were completed at Iowa State University, Ames, Iowa.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds.** Research on chemical composition and physical properties involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968); and to "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967).

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on

* Work covers more than one commodity; only effort allocated to wheat is included in total.

**Effort prorated among corn, wheat, and grain sorghum.

starch vinyl and epoxide graft copolymers (4 years, 1963-1967); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); Academy of Sciences and Chemical Institute "Boris Kidric," Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); and to the Institute for Fibres and Forest Products, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968). During the year, research was completed on fatty chemical derivatives of starch dextrins at the Institute of Industrial Chemistry, Bologna, Italy, and on changes induced in starch by gamma-irradiation at the National Institute of Agronomic Research, Paris, France.

Research on microbiology and fermentation involves grants to the University of Milan, Milan, Italy, for basic studies on the metabolic pathway to 5-ketogluconic acid in Acetobacter species (5 years, 1961-1966); University of Allahabad, Allahabad, India, for collection of new Mucorales species (5 years, 1961-1966), and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India to find new accessions for the ARS Culture Collection (5 years, 1965-1970); to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); to the University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and of mevalonic acid (3 years, 1965-1968); to the Institute of Biological Chemistry, University of Rome, Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966); to the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and to the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Characterization of wheat gluten proteins. Separation of whole gliadin on Sephadex columns yielded fractions of differing molecular weights (the low-molecular-weight fraction contains α-, β-, and γ-gliadins). Sedimentation studies on whole gliadin and on these fractions, intact and after reduction and alkylation, indicated the following weight-average molecular weights respectively: whole gliadin, 43,000 and 26,000; high-molecular-weight fraction, 126,000 and 37,000; low-molecular-weight fraction, 27,000 and 22,000.

Studies on the composition of γ-gliadin resulted in its fractionation into three major components. Each had a distinct amino acid composition, electrophoretic mobility, and chromatographic behavior. Unlike γ_2- and γ_3-gliadin, γ_1-gliadin was devoid of lysine and tryptophan and did not have aspartic acid as the N-terminal residue.

Glutenin was found to be more susceptible than gliadin to enzymic degradation. Behavior toward enzymes was consistent with the concept that disulfide bonds are intermolecular in glutenin and intramolecular in gliadin. The molecular weight of reduced alkylated γ-gliadin was found to be close to that of intact γ-gliadin in the same solvent system--a further indication of intramolecular disulfide bonding. Similar studies on reduced alkylated glutenin showed that its polypeptide subunits aggregated strongly even in powerful disaggregating solvents. The lowest apparent molecular weight for a subunit was 20,000. The reduction of disulfide bonds in glutenin could be controlled by use of less than stoichiometric amounts of mercaptoethanol and by variation of the pH of the reaction mixture. Further studies of optical rotatory dispersion indicated more α-helix in gliadin than in glutenin.

2. Desulfurization of wheat gluten proteins. Under the contract at Purdue University, further studies were conducted on the release of sulfur as sulfide at different levels of alkali and temperature, as well as in the presence of a reducing agent. The maximum yield of sulfide was around 15 percent of total sulfur at 25° C. but became about 75 percent at higher temperatures. In the presence of a reducing agent at 25° C., the liberation of sulfide from gliadin did not level off after 24 hours although it had reached a value of 75 percent of the cystine sulfur originally present.

Related research on cleavage of disulfide bonds in corn and wheat proteins is reported under Area 1, subheading A-3.

3. Chemistry of glycoprotein linkages. Under the grant to Marquette University on glycoproteins, study of a derivative of a glucosamine glycoside of serine showed that the glycoside carbohydrate moiety could

be removed by β-elimination with formation of derivatives of glucosamine and of α-aminoacrylic acid. The corresponding threonine derivative behaved similarly.

4. Microscopic and ultrastructure of wheat grain. Research on microscopic and ultrastructure of wheat grain and on changes induced therein by various treatments is relevant to industrial utilization of wheat. Results are reported under Area 5, subheading A-1.

5. NMR studies. Nuclear magnetic resonance techniques are employed in studies relevant to industrial utilization of wheat. Results are reported under Area 1, subheading A-4.

B. Chemical and Physical Investigations to Improve Products

1. Chemical modification of wheat gluten. Reduced whole gluten was chemically modified by graft copolymerization with methyl acrylate in dimethyl sulfoxide in the presence of sodium. The product was more soluble in organic solvents than the reduced gluten, but no longer dissolved in acid solutions. Studies on model compounds indicated that SH, NH_2, and $CONH_2$ groups reacted at about the same rate in graft copolymerizations of this type. Several S-alkyl cysteine derivatives were prepared as model compounds for study in connection with chemical modification of gluten.

Cyanoethylation of the amino group of tyrosine did not affect the pK of the phenolic group. This observation provided the basis for a novel differential potentiometric titration procedure for determination of amino and phenolic groups in aminophenols and in proteins. Terminally N-dicyanoethylated lysine was partially decarboxylated under conditions used for protein hydrolysis.

2. Polypeptide derivatives. Contract research at IIT Research Institute showed that sulfated polypeptides (obtained by hydrolysis of gluten) were less effective surfactants than some commercially available products. Polyether derivatives were prepared by reacting ethylene oxide with hydrolyzed gluten. By polymerization of acrylate monomers in the presence of these polyethers, products showing promise as adhesives were obtained. These polyethers also acted as plasticizers for films of some synthetic resins.

3. Chemical modification of wheat flour. Wheat flour reacted with ethylenimine (1.5 to 3 g./100 g. flour) imparted more strength to paper than did a commercial cationic sorghum flour, but slightly less than cationic corn starch.

4. Studies on wheat starch. Chemical and physical investigations on wheat starch are integrated with similar research on corn starch reported under Area 1, subheading B.

C. Microbiology and Fermentation

Research on microbiological and fermentative processes for converting wheat starch or flour to industrial products is integrated with similar studies on corn. Results are reported under Area 1, subheading C.

D. Technology - Process and Product Development

1. Milling and fractionation of wheat. Research on milling and fractionation of wheat is directed to production of improved products for industrial food and feed uses. Results are reported under Area 5, subheading C-1.

2. Acid-modified flour (AMF). In contract research at Iowa State University on fluidization of wheat flour, studies on HCl sorption rate were extended to fluidized beds where the rate was about 0.5 g./kg./min. from dilute concentrations of HCl in air. Ammonia was absorbed at about half the rate found for HCl. The specific heat of dry flour was determined to be 0.31 cal./g./°C. at 40-50°. At 12 percent moisture the value was 0.39. A continuous system for preparing AMF in a fluidized bed reactor was successfully operated. Products from soft white and hard red wheat flours had paste viscosities similar to those of products prepared conventionally at the Northern Division. This work essentially completes the activities specified in the contract.

Under a research contract, the Brown Company is evaluating AMF on a semi-commercial scale as a size for paper. Strength properties of paper sized with AMF were similar to those of paper treated with the reference size (a commercial hypochlorite-oxidized starch). During the sizing operations, which lasted about 2 hours, protein content doubled in the AMF pastes, but no interference with the operations or with properties of the paper produced was observed. Runs of longer duration are planned to determine if protein buildup reaches equilibrium or continues to increase.

3. Wheat-derived xanthates and xanthides in paper. In contract research at Battelle Memorial Institute, bran xanthate (D.S. 0.092) at the 10 percent level of addition increased strength properties of handsheets as follows: burst, 55 percent; dry tensile, 15 percent; and wet tensile, 1,100 percent. However, about 12 times the theoretical amounts of sodium hypochlorite was necessary to effect crosslinking of xanthate to xanthide. The cause for this excessive oxidant requirement is being sought. Gluten xanthate was ineffective as an additive.

4. Other developmental research. Development of products and processes involving wheat starch is integrated with related work on corn starch. Results are given under Area 1, subheading D.

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AREA NO. 5: WHEAT UTILIZATION - FOOD (NORTHERN REGION)

Problem. The dominant factor in the wheat economy of the United States continues to be a production capacity that can outpace consumption, including the substantially expanded foreign markets of recent years. Increased exports of wheat from the United States in the last 3 years have brought our wheat carryover to a level that provides less than a prudent reserve. However, the capacity to produce wheat in this country is still restricted.

We view this North American surplus capacity as an unparalleled opportunity. Wheat in excess of domestic needs can be used to buy time in the overpopulated areas of the world until a self-sufficient agriculture can be developed there. Export donations and concessional sales of 600-800 million bushels per year are providing food where it is most needed in the world. This distribution of wheat serves immediate Defense and State Department missions, and also stimulates a long-range market development for United States agriculture. New wheat foods specifically adapted to conditions of use in every region of the world would help materially to popularize this valuable food grain in areas where it is now virtually unknown, and development of simplified methods to process the products at the point of use would speed their adoption.

We also need to increase the commercial exports (currently less than 200 million bushels annually) that contribute favorably to our international trade balance. New processes to elicit maximum quality performance of wheats and flours in products produced in Europe and Japan would help significantly to promote trade in these dollar markets. Sustained further gains in wheat markets are necessary to ease governmental restrictions on production more than they have already been eased, and especially to strengthen export trade balances. Increased world supplies of wheat and restrictive political decisions in the European Economic Community have contributed to seriously reduced commercial exports in some years. Everything possible must be done to increase total wheat markets, but especially those in which payments are made in dollars.

Consumers of wheat foods in this country have benefited greatly by introduction of a wide variety of new and improved products. Well-balanced diets, reasonable food costs, and improved convenience result from such developments and are suitable objectives of research. Domestic per capita consumption has become stabilized over the past 3 years. Research programs along these lines thus sustain and increase markets for wheat.

An essential foundation for a successful product and process development program is basic research on the composition of all classes of wheat and the fundamental properties of their constituents. This kind of information provides the foundation for improved and new products and processes.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of wheat in food.

The Federal scientific effort (Northern region) for research on food utilization of wheat totals 11.2 scientist man-years. Of this number, 4.7 are devoted to chemical composition and physical properties; 4.7 to microbiology and toxicology; and 1.8 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (3.8 scientist man-years) includes studies on separation and physical and chemical characterization of wheat proteins and on the microscopic and ultrastructure of wheat grains and flours and the effects of various treatments. During the reporting period, a project on the effect of conditioning wheat on characteristics of milled fractions was completed and replaced by research on microscopic and ultrastructure.

A research contract (.9 scientist man-year) is in effect with the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on varietal variations in kernel properties and milling and fractionation characteristics of wheat.

Research at Peoria, Illinois, on microbiology and toxicology (4.7 scientist man-years) is concerned with development of new fermented foods from wheat, with reduction of the microbial population of wheat and wheat flour and with studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.2 scientist man-year) is allocated to research on food uses of wheat.

Research conducted at Peoria, Illinois, on technology - process and product development (1.8 scientist man-years) comprises investigations on new techniques for milling and fractionation of wheat to obtain improved products for food and other uses and on methods for reducing radioactive contamination of wheat and wheat flour.

The Department also sponsors research conducted by foreign institutions under grants of PL 480 funds. Research on technology - process and product development involves a grant to the Cereal Research Station, Research Association of British Flour-Millers, St. Albans, England, for investigations on quantitative measurement of properties of wheat that change significantly during conditioning (5 years, 1961-1966). This research was completed during the reporting period.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 22.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Microscopic and ultrastructure of wheat. Electron microscopy showed that lipoprotein at the protein-starch interface or in the wedge protein of wheat endosperm was substantially extracted with acetone, butanol-water, methanol-ethanol or methanol-diethylether. The extraction was more extensive for soft than for hard wheat. Removal of lipoprotein binds protein and starch and destroys dough-forming properties of flour. It was further shown by electron microscopy that wetting the aleurone layer during steeping of wheat led to extensive changes in cellular organization. This result is believed to be indicative of changes in the status of enzyme systems that originate in this layer.

2. Crosses of soft and hard wheats. Contract research at the University of Nebraska showed that softer lines of wheat exhibited poor milling properties but good air classification properties. The opposite was observed for harder lines. There was low correlation between hardness and protein content for identical lines grown in 1961 and 1964. In the protein of wheats characterized by lower protein content, there was some tendency toward increase of the nutritionally limiting amino acids (lysine, methionine, threonine). An improved liquid extraction procedure was developed for fractionation of endosperm protein.

3. Characterization of wheat gluten proteins. Results, reported under Area 4, subheading A-1, are important to understanding the unique functional properties of wheat flour.

4. Proteolysis inhibition by cereal flours and starches. This research was completed last year. The final report, which was received subsequently, provided additional important information about the water-soluble trypsin inhibitor (TI) that was found in the flours of cereal grains. In order of decreasing TI activity, the cereals studied were buckwheat, rye, wheat, barley, corn, oats, and rice. The concentration of TI in buckwheat was about 1/15 that of soybeans, and the activity of rice was about 1/175 of the activity of soybeans. Studies on the isolation of trypsin inhibitor resulted in a procedure for concentrating the activity. Electrophoretic analysis yielded two fractions with antitryptic activity. Both fractions were peptides (or proteins).

Cooking completely destroyed TI in wheat, rice, and millet and strongly reduced TI in oats, but did not affect TI in buckwheat, rye, barley, and corn. Thus, wheat bread had no TI, but some was retained in rye bread. Malted barley retained activity. The incubation of corn at pH 12 destroyed TI.

The mode of action of TI appeared to be similar for all of the cereals and to involve a strong bonding of the inhibitor to its site of action. Studies on the physiological activity of TI concentrates showed that protein digestion in rats was impaired by high levels of inhibitor equivalent to a greater weight of flour than could be eaten by the animal. Time did not permit the investigators to establish whether destruction of inhibitor would be beneficial for animals on a high-cereal diet. Likewise, complete studies on the laxative effect of TI (or accompanying constituents) were not possible. Relative to human consumption of cereal grain products, any effect of trypsin inhibitor seems very unlikely in view of the relatively low levels of inhibitor activity.

B. Microbiology and Toxicology

1. Reduction of viable microorganisms in flour and flour products. Studies on reducing microbial population of wheat flour showed that heating wheat before milling for 6 hours at 60° C. and 13 percent moisture, in combination with use of chlorine in temper and wash waters, yielded a final flour having less than 1 percent of the microbial count of flour from untreated wheat. Functional properties of the flour were not damaged. This process, which avoids problems and disadvantages encountered in direct treatments of flour, is an attractive means for producing flours suitable for use in uncooked frozen or refrigerated foods and for baby foods and other canned products containing flour.

Examination of 1965 crop wheats and flour samples from 22 representative mills in the Montana-North Dakota and Michigan-Indiana study areas showed that psychrophiles constitute the major part of the bacterial flora of wheat and flour, accounting for as much as 94 percent of the bacterial population in wheat and 70 percent in flour.

2. Fermented wheat foods. Studies on wheat tempeh showed that it retains a large part of the nutritive values of whole wheat and is also enriched with niacin and riboflavin as a result of the action of the fermenting organism Rhizopus oligosporus. Losses in making wheat tempeh were about 8 percent as a result of washing, cooking, and draining, whereas losses from fermentation ranged from 3.3 to 27.3 percent depending on length of incubation. On a dry solid basis, recovery is 88.5 to 64.5 percent. Since losses are mainly in carbohydrate and carbohydrate-like materials, the percentage of protein in tempeh is increased over that in the wheat substrate.

Initial research on products resembling sufu (Chinese cheese) revealed that brining of the fermented substrate, besides inhibiting mold growth and imparting a salty taste, serves also to elute protease from the mold mycelium and thus enables the enzyme to act on protein in the substrate. Neutral compounds cannot replace NaCl or other salts for this purpose, a fact

suggesting that protease may be bound to the mycelium surface by a weak ionic linkage. Two molds used in making sufu grew and released proteases into a medium containing wheat flour and NaCl.

3. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of wheat in food. Results are reported under Area 3, subheading B-2.

C. Technology - Process and Product Development

1. Milling and fractionation. Fine grinding and air classification of a new variety of Gaines soft wheat ("Nugaines") resulted in a protein shift of 70 percent as compared to 77-80 percent for the regular variety. Barley was found to resemble soft red winter wheat in its response to fine grinding and air classification. Methods were developed for preparation of ground whole wheat and SWW wheat bran having particle sizes equivalent to that of conventional HRW wheat flour. These products are suitable for evaluation as additives for paper either with or without chemical modification.

2. Reduction of radioactive contamination of wheat and milled products. Phosphoric acid was shown to be effective for removing strontium-90 from wheat. As finally optimized, the process requires washing the grain for 3 to 4 hours at room temperature in 0.1 to 0.2 percent phosphoric acid solution followed by rinsing, centrifuging, and drying the grain. The grain can then be conventionally tempered and milled. Washing time may be reduced at least 1 hour by employing mechanical mixers. The used phosphoric acid solution may be decontaminated for re-use with little or no loss of phosphoric acid content by passing it through the cation resin Amberlite IR-120. Other acids such as hydrochloric, nitric, or citric may be used with similar results if phosphoric acid is not available. When field-grown wheat with a relatively low initial strontium-90 content (395 pCi/kg) was treated by this method, a reduction of strontium-90 in the wheat of 73 percent was obtained. This was reflected in reductions of 76 percent in bran, 49 percent in shorts, and 21 percent in the clear and patent flours. Use of fine grinding and air classification resulted in further reduction in radioactivity of flour. These studies have progressed as far as is practical with available materials. Any further useful extension of this work would require simulation of highly contaminated wheat samples such as would be anticipated from massive radioactive fallout. This research, which was carried out with the cooperation of the Health and Safety Laboratory of the AEC, has accordingly been terminated.

3. Quantitative measurement of wheat conditioning variables. This research is being conducted under a PL 480 grant to the Research Association of British Flour-Millers, St. Albans, England. Results showed that tempering moisture has a marked effect on endosperm breakdown into particles below

35 μ in diameter and that the response varies with the type of stress applied. Thus, in roller milling, breakdown is greater at high moisture levels; whereas in impact milling, breakdown increases at low endosperm moisture. A subaleurone fraction with an average protein content of 44 percent and making up about 5 percent of the flour was recovered from coarse air-classified flour.

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AREA NO. 6: WHEAT UTILIZATION - FEED (NORTHERN REGION)

Problem. In the last 2 years the use of wheat for feed increased to nearly 100 million bushels per year, more than twice the amount used in any other recent year. Unfortunately, wheat has certain performance drawbacks as a feed. Research that develops new processes to improve feeding quality of wheat will benefit both growers and feeders in wheat-producing areas, since it will place this grain in a more competitive position as compared with other grains, and it can reduce freight costs.

Millfeeds are not used extensively in modern poultry and swine rations because the high fiber content cannot be tolerated in high-energy rations. If inexpensive ways of separating low-fiber, high-protein fractions from millfeeds are developed, these new materials can be used as protein and energy sources for nonruminant diets, and the overall value of milling byproducts will be increased. Flour production is expected to increase in the near future to reflect the demands of our increasing domestic population and of the new export markets which are developing. More milling will result, of course, in more millfeeds. If these millfeeds cannot be utilized efficiently and effectively, the price for flour will have to increase to carry the economic burden.

Meat production, particularly poultry, is increasing rapidly in Japan and the European Economic Community where modern efficient methods have been introduced. This development depresses the opportunity for exporting poultry and other meats into these important trade areas, but it offers an increasing opportunity to sell feeds. Upgrading of wheat millfeeds through utilization research will increase our export markets.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of wheat in feed.

The Federal scientific effort (Northern region) for research on utilization of wheat in feeds totals 3.8 scientist man-years, of which .9 is devoted to chemical composition and physical properties; 2.1 to microbiology and toxicology; and .8 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (.9 scientist man-year) involves basic investigations of the microscopic and ultrastructure of wheat grains and the effects of various treatments. During the year, research on the effects of wheat conditioning and characteristics of milled fractions was completed.

Research at Peoria, Illinois, on microbiology and toxicology (1.3 scientist man-years) is concerned with studies on the production of mycotoxins by Aspergillus flavus and related molds. Research contracts (.8 scientist man-year*) are in effect with A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene; with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene; and with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites.

Research conducted at Peoria, Illinois, on technology - process and product development (.8 scientist man-year) is concerned with studies on new techniques of milling and fractionation to obtain improved products and with development of methods for minimizing radioactive contamination of wheat and milled fractions.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains. Effort on this project is prorated among corn, wheat, and sorghum. During the reporting period, research was completed at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, on production of Vitamin B_{13} and at the National Institute for Agronomic Research, Paris, France, on mutation of yeasts for improved feeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

Studies on the microscopic and ultrastructure of wheat are relevant to utilization of wheat in feeds. Results are reported under Area 5, subheading A-1.

B. Microbiology and Fermentation

1. Microbial carotenoids. Research on fermentative conversion of cereal grains to carotenoid-rich additives for feeds is applicable to wheat. Results are reported under Area 3, subheading B-1.

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*Work covers more than one commodity; only effort allocated to wheat is included in total.

2. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of wheat in feeds. Results are reported under Area 3, subheading B-2.

3. Vitamin B_{13}. Studies on Vitamin B_{13} are pertinent to utilization of wheat in feeds. Results are reported under Area 3, subheading B-3 (PL 480 research).

4. Improved feeds by mutation of yeasts. Studies on mutant yeasts capable of producing high yields of sulfur-containing amino acids are relevant to feed utilization of wheat. Results are reported under Area 3, subheading B-4 (PL 480 research).

C. Technology - Process and Product Development

Research on milling and fractionation of wheat and on reduction of radioactive contamination in wheat and milled products is relevant to utilization of wheat in feeds. Results are reported under Area 5, subheading C.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

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AREA NO. 7: GRAIN SORGHUM UTILIZATION
INDUSTRIAL PRODUCTS

Problem. The growing importance of grain sorghum as a competitive crop is revealed by the record 655-million-bushel crop in 1965. About 80 percent of the grain sorghum crop is grown in Texas, Kansas, and Nebraska. Sorghum starch and flour find industrial usage where freight transportation advantages exist. Currently an estimated 5 million bushels of sorghum are milled for products consumed mainly by the paper and gypsum board industries. To maintain this market against competition from synthetics and to take advantage of opportunities that exist in economically favorable geographic areas for increased industrial utilization of sorghum, technology suited to the specific characteristics of this grain and its milled products must be developed.

Research on sorghum starch is integrated with that on corn starch. A more detailed discussion of promising industrial outlets and of the pertinent research required is given under Area 1, Corn Utilization - Industrial Products. Because grain sorghum has a round kernel in contrast to the odd-shaped kernel of corn, it lends itself to dry milling innovations not possible with corn. Milling improvements, together with the possible advantages of air classification of sorghum flour, offer prospects for increasing industrial utilization by making processing economics more attractive and by providing products with superior properties.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies on the composition of grain sorghum, on characterization and properties of the components, and on their chemical and microbiological conversion to useful industrial products.

The Federal scientific effort for research on industrial utilization of grain sorghum totals 9.1 scientist man-years. Of this number, .9 is devoted to chemical composition, physical properties and structure; 3.3 to chemical and physical investigations to improve products; 3.0 to microbiology and fermentation; and 1.9 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.2 scientist man-year) involves study of applications of nuclear magnetic resonance spectroscopy to grain components and is integrated with related research on corn.

A research contract is in effect with Indiana University Foundation, Bloomington, Indiana, for studies on the isolation and characterization of

phenolic pigments of grain sorghum (.6 scientist man-year). A grant (.1 scientist man-year*) to Iowa State University, Ames, Iowa, provides for basic research on heat, mass, and momentum transport of cereal starches and flours.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (2.4 scientist man-years) is integrated with research on corn starch and is directed to wide-ranging study of the chemical reactions of starch with the objective of discovering new chemical products and processes having potential for industrial use. During the year, one phase of this work involving study of possible means for preparing amino acid derivatives of starch was completed and replaced by research on synthesis of halogen derivatives of starch. Research contracts (.4 scientist man-year*) are in effect with the Arizona Agricultural Experiment Station, University of Arizona, Tucson, Arizona, for basic studies on the reaction of acetylene with methyl glucoside; with The Johns Hopkins University, Baltimore, Maryland, for basic research on the reactions of starch in fluid dynamic media; with University of Pittsburgh, Pittsburgh, Pennsylvania, for studies on dielectric activation of starch; with Southern Illinois University, Carbondale, Illinois, for investigations on synthesis of maltooligosaccharides; with Stanford Research Institute, Menlo Park, California, for research on graft copolymers of cereal starches with vinyl-type monomers; and with the Institute of Paper Chemistry, Appleton, Wisconsin, for investigation of physical chemical factors affecting retention and effectiveness of starch xanthates and xanthides in paper. Contract research was completed by Ohio State University, Columbus, Ohio, on synthesis of amino derivatives of starch and by the University of Arizona Agricultural Experiment Station, Tucson, Arizona, on the reaction of starch with mercaptans. Grants (.5 scientist man-year*) have been made to Ohio State University Research Foundation, Columbus, Ohio, for basic research on the reaction of vinyl ethers with carbohydrates; to Ohio State University, Columbus, Ohio, for basic investigations of unsaturated and sulfur-containing carbohydrates and of the amination of starch; to Purdue Research Foundation, Lafayette, Indiana, for studies on sugars containing carbon-bound nitrogen, phosphorus and sulfur; and to the University of Arizona, Tucson, Arizona, for basic research on the reaction of starch with diepoxides.

Research on microbiology and fermentation conducted at Peoria, Illinois (2.6 scientist man-years), includes studies on the use of microorganisms to convert cereal-based media to industrially useful products such as chemicals, enzymes, polymers, and biological insecticides. This research is integrated with similar studies based on corn. A large collection of pure cultures of industrially and agriculturally important microorganisms is maintained. The Pioneering Laboratory for Microbiological Chemistry

*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

conducts research on microbiological reactions and products. Investigations on biological insecticides for Japanese beetle and on other insect control agents is cooperative with Entomology Research Division and Plant Pest Control Division. Research on plant antibiotics involves cooperation with Crops Research Division. Research contracts (.1 scientist man-year*) are in effect at Michigan State University, East Lansing, Michigan, for basic research on enzyme activity in sporulation; at Kansas State University, Manhattan, Kansas, for investigation of stabilization of vegetative cells of the pathogenic organisms; at the University of Minnesota, St. Paul, Minnesota, for fundamental studies on the transfer of genetic determinants of sporulation from one microorganism to another; at Baylor University, Houston, Texas, for investigation of morphological changes involved in sporulation; and at the American Type Culture Collection, Rockville, Maryland, for studies on preservation of certain microorganisms for which lyophilization is ineffective. Contract research at the University of Illinois, Urbana, Illinois, for research on the applicability of a sporulation factor produced by bacteria to Japanese beetle pathogens has been completed. Grants (.3 scientist man-year*) have been made to Cornell University, Ithaca, New York, for fundamental studies on biphasic fermentation; to the Nebraska Agricultural Experiment Station, University of Nebraska, Lincoln, Nebraska, for investigations on the nature of amylase enzymes; to Kansas State University, Manhattan, Kansas, for investigations on separation of enzymes and proteins by disc electrophoresis; to Iowa State University, Ames, Iowa, for investigation on bacterial amylases and their action patterns; to the University of Wisconsin, Madison, Wisconsin, for studies on the fine structure of polysaccharide B-1973; and to the University of Arkansas, Fayetteville, Arkansas, for investigation of the mechanism of enzymatic hydrolysis of starch.

Research conducted at Peoria, Illinois, on technology - process and product development (1.2 scientist man-years) is concerned with detailed study and evaluation of starch derivatives having definite potential for industrial utilization and of processes for making them. The work is integrated with similar studies on corn starch derivatives. Research contracts (.7 scientist man-year*) are in effect with Stanford Research Institute, Menlo Park, California, for process development of selected starch graft copolymers; with Battelle Memorial Institute, Columbus, Ohio, for developmental research on starch and other cereal grain xanthides and for studies on starch derivatives for use as colloids in water-emulsion paints; with Western Michigan University, Kalamazoo, Michigan, for evaluation of modified cyanoethylated starches for applications in paper; with Archer Daniels Midland Company, Minneapolis, Minnesota, for investigations on the use of starch glycosides in coatings and plastics; and with University of Akron, Akron, Ohio, for evaluation of starch and starch derivatives as reinforcing agents for

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*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

natural and synthetic rubber. During the year, contract research on evaluation of starch polyol urethane foams was completed by Archer Daniels Midland Company, Minneapolis, Minnesota.

The Department also sponsors research on cereal starches conducted by foreign institutions under grants of PL 480 funds.* Research on chemical composition, physical properties and structure involves grants to the University of London, London, England, for research on debranching enzymes and their use in studying the fine structure of starch components (5 years, 1963-1968); to the University of Osaka Prefecture, Sakai, Japan, for development of an analytical method for carbonyl groups in carbohydrates (4 years, 1964-1968); and to "Giuliana Ronzoni" Scientific Institute for Chemistry and Biochemistry, Milan, Italy, for research on glucopyranose rings in starches and dextrins (5 years, 1962-1967).

Research on chemical and physical investigations to improve products involves grants to Hebrew University, Jerusalem, Israel, for studies on starch vinyl and epoxide graft copolymers (4 years, 1963-1967); National Institute of Technology, Rio de Janeiro, Brazil, for research on phosphorus- and sulfur-containing cationic starches (5 years, 1962-1967); Ahmedabad Textile Industry's Research Association, Ahmedabad, India, for research on starch-gum copolymers prepared by codextrinization (5 years, 1963-1968), and for studies on preparation and characterization of hydroxyethyl ethers of cereal starches (5 years, 1965-1970); Academy of Sciences and Chemical Institute "Boris Kidric", Ljubljana, Yugoslavia, for studies on modification of starch by moisture and temperature treatments (5 years, 1964-1969); Plastics Research Institute TNO, Delft, The Netherlands, for research on preparation of metal alkoxides of starch for use as intermediates in synthesis (5 years, 1964-1969); and University of Edinburgh, Edinburgh, Scotland, for studies on the mechanism and structural changes involved in thermal, acid, and alkaline degradation of starches (5 years, 1964-1969); and to the Institute of Fibres and Forest Products Research, Jerusalem, Israel, for studies on the mechanism and products of mild oxidation of starch (5 years, 1963-1968). During the year, research was completed on fatty chemical derivatives of starch dextrins at the Institute of Industrial Chemistry, Bologna, Italy, and on changes induced in starch by gamma-irradiation at the National Institute of Agronomic Research, Paris, France.

Research on microbiology and fermentation involves grants to the University of Milan, Milan, Italy, for basic studies on the metabolic pathway to 5-ketogluconic acid in Acetobacter species (5 years, 1961-1966); University of Allahabad, Allahabad, India, for collection of new Mucorales species (5 years, 1961-1966), and studies on survival of lyophilized microorganisms (5 years, 1962-1967); University of Newcastle upon Tyne (formerly University of Durham), Newcastle upon Tyne, England, for investigations of sugar phosphate derivatives in molds (5 years, 1962-1967); Central Drug Research Institute, Lucknow, India, for studies on aerobic actinomycetes in India to

*Effort prorated among corn, wheat, and grain sorghum.

find new accessions for the ARS Culture Collection (5 years, 1965-1970); to the University of Liege, Liege, Belgium, for research to find lytic enzymes of microbial origin (5 years, 1964-1969); to the University of Lodz, Lodz, Poland, for research on the fermentative production of itatartaric acid (5 years, 1963-1968); University of Tokyo, Tokyo, Japan, for research on the fermentative production of D-tartaric acid (5 years, 1964-1969) and of mevalonic acid (3 years, 1965-1968); to the Institute of Biological Chemistry, University of Rome, Rome, Italy, for studies on the preparation and characterization of dextran derivatives (5 years, 1961-1966); to the National Sugar Institute, Kanpur, India, for research on isolation of natural polysaccharide gums (3 years, 1965-1968); and to the National Institute of Agronomic Investigations, Madrid, Spain, for study and collection of aerobic species of actinomycetes (4 years, 1965-1969).

PROGRAM OF STATE EXPERIMENT STATIONS

State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Phenolic pigments of grain sorghum. In contract research at Indiana University, methanol extraction was found to remove pigments from dewaxed sorghum pearlings. Six or more leucoanthocyanidins or leucoanthocyanins were detected in the methanol extract of dewaxed sorghum pearlings. Also, treatment of this methanol extract with lead acetate yielded five or more substances of the coumarin or cinnamate type. Methods for characterizing phenolic pigments in the extracts are being investigated.

2. NMR studies. Nuclear magnetic resonance techniques are employed in studies relevant to industrial utilization of grain sorghum. Results are reported under Area 1, subheading A-4.

B. Chemical and Physical Investigations to Improve Products
C. Microbiology and Fermentation
D. Technology - Process and Product Development

Research in these categories is integrated with similar investigations on corn starch. Results are reported in Area 1, subheadings B, C, and D.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition, Physical Properties and Structure

Blessin, C. W., and Dimler, R. J. 1966. Current grain sorghum research. Sorghum Newsletter 9, pp. 16-17.

Dimler, R. J. 1965. Utilization research on grain sorghum. Proc. Fourth Grain Sorghum Research and Utilization Conf., Amarillo, Texas, Feb. 25-26, 1965, pp. 17-20.

Technology - Process and Product Development

Weinecke, L. A., and Montgomery, R. R. 1965. Experimental unit now suitable for scale-up to mill production size. Am. Miller 93(9), pp. 8-9, 33.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

None.

AREA NO. 8: GRAIN SORGHUM UTILIZATION - FOOD

Problem. An estimated 6 million bushels of grain sorghum are utilized annually in products for human consumption. This sorghum includes some special varieties such as white and waxy sorghums. Sorghum starch and derived glucose and glucose sirup are used in foods, and sorghum grits are used in fermented beverages. Although this outlet is at present quite limited, the growing importance of grain sorghum as a cash crop in the Southwest indicates that opportunities for increasing food use of sorghum should not be overlooked. Since grain sorghum is a staple food in many parts of Asia and Africa, a further consideration is the development of food products that could contribute to alleviation of dietary deficiencies in many developing countries.

To achieve the objective, more information on the composition of grain sorghum is needed. For example, some varieties contain pigments that can discolor milled products and that may contribute to undesired flavors. Questions have been raised concerning the digestibility and nutritive value of sorghum protein that reveal the need for better data on amino acid composition and on minor constituents.

Milling innovations, such as tangential abrasion, make possible conversion of about 20 percent of the sorghum kernel to a flour containing 25 percent protein. This and other possible approaches to new food products should be evaluated.

It has recently been discovered that certain oilseeds and cereal grains, including sorghum, are subject to infection by molds that can produce toxic products. To provide safe food products, as well as to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from sorghum.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of grain sorghum in food.

The Federal scientific effort for research on food utilization of grain sorghum totals .7 scientist man-year. Of this number, .3 is devoted to chemical composition and physical properties and .4 to microbiology and toxicology.

Research on chemical composition and physical properties involves a contract with Kansas State University, Manhattan, Kansas, for investigations on the

composition, processing, and feeding value of hybrid grain sorghum. A portion of this effort (.3 scientist man-year) is allocated to research on food uses of grain sorghum.

Research at Peoria, Illinois, on microbiology and toxicology (.3 scientist man-year) is devoted to studies on the production of mycotoxins by Aspergillus flavus and other molds. The work also includes a survey of the incidence of aflatoxin in commercial samples of various grains. A research contract in effect with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, provides for a survey of various species of Aspergilli to find and identify those producing toxic metabolites. A portion of this effort (.1 scientist man-year) is allocated to research on food uses of grain sorghum.

PROGRAM OF STATE EXPERIMENT STATIONS

State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nutritional quality of grain sorghum. Contract studies on composition, processing, and feeding value of hybrid grain sorghums are important to food utilization of sorghum. Results are reported under Area 9, subheading A-1.

B. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of grain sorghum in foods. Results are reported under Area 3, subheading B-2.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

None.

AREA NO. 9: GRAIN SORGHUM UTILIZATION - FEED

Problem. The principal domestic use of grain sorghum produced in the U. S. is as feed for animals. The record 655-million-bushel crop in 1965 reveals the growing importance of this grain. About 80 percent of the crop is grown in Texas, Kansas, and Nebraska.

Problems are encountered in the use of grain sorghum in feeds which, if solved, could increase utilization and economic value of this crop to farmers and to the feed industry. The major need is for more and better information on the protein content and amino acid composition of various varieties of grain sorghum as related to biological feeding value. Minor constituents having physiological activity also require more adequate study. For example, certain phenolic pigments may impart bitterness and thereby reduce palatability. Carotenoid pigments, which in part are precursors for Vitamin A, are valuable in poultry rations for imparting yellow color to egg yolks and to the skin of fryers and broilers. In addition to such compositional studies, processing investigations are needed to provide ways for preserving desired and removing undesired components. Sorghum is included in the group of cereal grains and oilseeds recently recognized to be subject to infection by molds capable of producing toxic products. To provide safe feed products and to minimize economic losses, research is needed on the detection of these toxins; on their quantitative analytical determination; and on development of processing techniques for their detoxification or removal from grain sorghum.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical, organic, and physical chemists, biochemists, microbiologists, systematic biologists, and chemical engineers engaged in basic, applied, and developmental studies pertinent to utilization of grain sorghum in feed.

The Federal scientific effort for research on utilization of grain sorghum in feed totals 1.2 scientist man-years, of which .7 is devoted to chemical composition and physical properties and .5 to microbiology and toxicology.

Research at Peoria, Illinois, on chemical composition and physical properties involved study of carotenoid pigments of grain sorghum and has been completed. A research contract is in effect with Kansas State University, Manhattan, Kansas, for investigations on the composition, processing, and feeding value of hybrid grain sorghums. A portion of this effort (.7 scientist man-year) is allocated to research on feed uses of grain sorghum.

Research at Peoria, Illinois, on microbiology and toxicology (.3 scientist man-year) is concerned with studies on the production of mycotoxins by

Aspergillus flavus and related molds. Research contracts (.2 scientist man-year*) are in effect with A. D. Little, Inc., Cambridge, Massachusetts, for studies on stabilization of fermentative β-carotene; with Consolidated Laboratories, Inc., Chicago Heights, Illinois, for research on the use of antimetabolites to facilitate selection of higher yielding strains of microorganisms producing β-carotene; and with the Agricultural Experiment Station, South Dakota State University, Brookings, South Dakota, for survey of the genus Aspergillus to find and identify species producing toxic metabolites.

The Department also sponsors research in this area conducted under grants of PL 480 funds. Research on chemical composition and physical properties involves a grant to the Indian Institute of Science, Bangalore, India, for research on separation of grain sorghum proteins (5 years, 1963-1968).

Research on microbiology and toxicology involves a grant to the Agricultural University, Poznan, Poland, for studies to increase the yield of β-carotene produced by fermentation of cereal grains. Effort on this project is prorated among corn, wheat, and sorghum. During the reporting period, research was completed at the "Giuliana Ronzoni" Scientific Institute of Chemistry and Biochemistry, Milan, Italy, on production of Vitamin B_{13} and at the National Institute for Agronomic Research, Paris, France, on mutation of yeasts for improved feeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 3.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Nutritional quality of grain sorghum. Preliminary results of contract research at Kansas State University showed that as protein level increases within a sorghum hybrid, all amino acids increase on an absolute weight basis. The relative amount of lysine, histidine, arginine, threonine, and glycine decreased, however, whereas that of glutamic acid, proline, alanine, and leucine increased. Apparently because of extremely favorable growing conditions in 1965, sorghum hybrids expected to exhibit the various levels of protein consistently observed in 1961, 1962, and 1963, instead exhibited essentially uniform protein levels.

2. Studies on sorghum proteins. At the Indian Institute of Science, qualitative differences were observed among disc electrophoresis patterns in polyacrylamide gels of protein fractions extracted with water, 1 percent NaCl, and 60 percent alcohol at 60° C. from three Indian varieties of sorghum. Fractions are being further characterized by gel electrophoresis after

*Work covers more than one commodity; only effort allocated to grain sorghum is included in total.

sulfide bond modification by reduction or by oxidation. One component of the alcohol-soluble protein fraction appears to contain no cystine. Experiments are in progress on the estimation of lysine present in protein hydrolyzates of about 40 varieties of sorghum seed of world-wide origin. This research is being conducted under a PL 480 grant.

B. Microbiology and Toxicology

1. Microbial carotenoids. Research on fermentative conversion of cereal grains to carotenoid-rich additives for feeds is applicable to grain sorghum. Results are reported under Area 3, subheading B-1.

2. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of grain sorghum in feeds. Results are reported under Area 3, subheading B-2.

3. Vitamin B_{13}. Studies on Vitamin B_{13} are pertinent to utilization of grain sorghum in feeds. Results are reported under Area 3, subheading B-3 (PL 480 research).

4. Improved feeds by mutation of yeasts. Studies on mutant yeasts capable of producing high yields of sulfur-containing amino acids are relevant to feed utilization of grain sorghum. Results are reported under Area 3, subheading B-4 (PL 480 research).

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

Chemical Composition and Physical Properties

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Deyoe, C. W., and Shellenberger, J. A. 1965. Amino acids and proteins in sorghum grain (nutritive value of grains). J. Agr. Food Chem. 13(5), pp. 446-450. (Kans.)

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Garrett, W. N., and Worker, G. F., Jr. 1965. Comparative feeding value of silage made from sweet and dual purpose varieties of sorghum. J. Animal Sci. 24, p. 782. (Calif.)

Mann, H. O., and Doherty, T. J. 1965. Sudan, sorghum-sudan hybrid yield and quality test. Colo. Agr. Exp. Sta. Progr. Rep. 175, pp. 1-2. (Colo.)

Shane, J. F., and Link, L. A. 1965. Results of the Kentucky grain sorghum performance test, 1964. Ky. Agr. Exp. Sta. Progr. Rep. 142, pp. 1-7. (Ky.)

Thayer, R. H. 1965. Grain sorghums in commercial rations for broilers, turkeys, and laying hens. Okla. Agr. Exp. Sta. Process. Ser. P-505, pp. 1-4. (Okla.)

Voigt, R. L., and Baker, E. A. 1965. Arizona grain sorghum, forage sorghum and sudan-grass performance tests, 1964. Ariz. Agr. Exp. Sta. Rep. 226, pp. 1-22. (Ariz.)

Walter, T. L. 1965. 1964 Kansas grain sorghum performance tests. Kans. Agr. Exp. Sta. Bull. 480, pp. 1-46. (Kans.)

AREA NO. 10: SOYBEAN UTILIZATION
INDUSTRIAL PRODUCTS

Problem. As an industrial oil, soybean oil is faced with growing competition from synthetic products derived from nonagricultural sources. As an industrial source of linoleic acid, soybean fatty acids must also compete with tall oil fatty acids, a byproduct of paper manufacture. Largely because of effective research, nonfood usage of soybean oil has rather consistently accounted for about 10 percent of domestic disappearance. The best opportunity for maintaining or increasing industrial applications of soybean oil is to be found in development of nontraditional products that can compete with synthetics in the multibillion-pound market for resins, fibers, coatings, plastics, plasticizers, pesticides, and paper and textile chemicals. To achieve the objective, more fundamental information is needed on reactions of soybean oil, especially those that will preserve the glyceride structure, and on the physical and chemical properties of the products.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing, long-range program involving analytical, organic, and physical chemists, and chemical engineers engaged in basic and applied research to obtain new information on chemical reactions of soybean oil and its components and to use this information to develop new or improved products for use by the chemical and other industries. In addition, microbiologists are engaged in a limited study of the possibilities of fermentative modification of fatty acids derived from soybean oil.

The Federal scientific effort for research on industrial utilization of soybean oil totals 13.7 scientist man-years. Of this number, .8 is devoted to chemical composition, physical properties and structure; 7.5 to chemical and physical investigations to improve products; 1.7 to microbiology and fermentation; and 3.7 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.8 scientist man-year) is devoted to mass spectrometric investigations of chemical and molecular structure of glyceride oils and their derivatives.

Research on chemical and physical investigations to improve products in progress at Peoria, Illinois (6.8 scientist man-years), emphasizes studies of aldehyde derivatives of soybean oil. A research contract with the University of Illinois, Urbana, Illinois, provides for basic studies on the mechanism of homogeneous hydrogenation with organometallic catalysts. A portion of this effort is allocated to industrial utilization of soybean oil (.4 scientist man-year). Also, a research contract with North Dakota State

University of Agriculture and Applied Science, Fargo, North Dakota, provides for investigations of aldehyde oils as components of protective coatings (.3 scientist man-year*).

Research at Peoria, Illinois, on microbiology and fermentation (1.7 scientist man-years) involves exploration of possibilities for producing industrially useful derivatives by microbial conversion of fatty acids.

Research at Peoria, Illinois, on technology - process and product development involved engineering studies on production of aldehyde oils from soybean oil (1.4 scientist man-years). This work was completed by the close of the reporting year. Research contracts (2.3 scientist man-years*) are in effect with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils; and with Archer Daniels Midland Company, Minneapolis, Minnesota, for pilot preparation of various aldehyde oil products needed for developmental investigations.

The Department also sponsors research in this area under grants of PL 480 funds to foreign institutions. Effort is prorated between soybean and linseed oils. Chemical and physical investigations to improve products are pursued under a grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on stereospecific polymerization of polyunsaturated fatty esters (2 years, 1965-1967). During the year, research was completed on alkaline cleavage of polyunsaturated fatty acids at Queen Mary College, University of London, London, England; and on oxidation with atmospheric oxygen to obtain new linseed and soybean oil derivatives at the Experiment Station for the Fats and Oils Industry, Milan, Italy.

Research on microbiology and toxicology involves a grant to the University of Baroda, Baroda, India, for studies on production of microbial lipases useful for modifying vegetable oils (5 years, 1965-1970).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Mass spectroscopy. Mass spectrometric investigations of chemical and molecular structure of glyceride oils and their derivatives are relevant to industrial utilization of soybean oil. Results are reported under Area 11, subheading A-1.

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*Work covers more than one commodity; only effort allocated to soybeans is included in total.

B. Chemical and Physical Investigations to Improve Products

1. Oxidative cleavage of unsaturated fatty acids. Studies were conducted on two novel and potentially practical ozonization methods. Thermal decomposition of ozonolysis products in water gave 40-50 percent yields of aldehyde. Reductive decomposition in the presence of propylene appeared to form no propylene oxide and gave aldehyde yields equivalent to those in its absence.

Optimum ozonolysis conditions developed for preparing methyl azelaaldehydate from methyl oleate were not directly applicable to methyl erucate because the erucate and the expected methyl brassylaldehydate are less soluble in the reaction solvent. Preliminary results with erucic acid, which is more soluble in acetic acid/butanol, were encouraging.

2. Aldehyde oil derivatives. Studies during the past year reflect the versatility of the aldehyde group for derivatization and have significantly augmented the type and number of potentially useful products obtainable from soybean and other oils via the ozonization route.

Either secondary or tertiary amines were prepared in yields of 80-85 percent by reductive alkylation of ammonia with pelargonaldehyde and methyl azelaaldehyde (MAZ). Type of product is determined by choice of catalyst and solvent system. 9-Aminononanamide, a potential source of tough polyamides having low water absorption, was prepared in over 30-percent isolated yield by simultaneous amination and ammonolysis of MAZ. Methyl brassylaldehydate could be converted to methyl 13-aminotridecanoate in excellent yields. Bis(8-carbomethoxyoctyl)amine resulted in 42-percent isolated yield from amination of MAZ under anhydrous conditions. Its N-acetyl derivative is a liquid that does not solidify at -70° C.

Bulk polymerization of MAZ glycerol acetal with calcium oxide catalyst gave polymers with molecular weights of 7,000 to 14,460 and melting points of 40 to over 100° C. Some of these polymers may have potential as elastomers. Two of the four possible isomers of MAZ glycerol acetal were isolated as crystalline solids and characterized.

Zinc acetate and lead octoate were the most effective of 11 catalysts tested for formation of crosslinked adherent films on glass from poly(ester-acetals) and poly(amide-acetals) derived from the pentaerythritol acetal of MAZ. Temperatures above 275° C. and times exceeding 30 minutes were, however, required for film formation. The films had good resistance to water, organic solvents, and 5 percent hydrochloric acid. The poly(ester-acetal) films were degraded by alkali whereas the poly(amide-acetal) films were more resistant.

Soybean oil ozonolysis products were effective peroxidic initiators for crosslinking of unsaturated polyester resins. Preliminary test samples showed both advantages and disadvantages in comparison to controls cured with benzoyl peroxide.

Polymethylol compounds were prepared in 90-percent crude yields by alkaline condensation of formaldehyde with hexanal or nonanal. These aldehydes result from ozonolysis of linoleate and oleate, respectively. Compounds of this type have applications in alkyd resins and as plasticizers and lubricants.

3. Cyclic fatty acids. Studies on the preparation of cyclic fatty acids are relevant to industrial utilization of soybean oil. Results are reported under Area 12, subheading C-1.

4. New chemical products. Research under the PL 480 grants to Queen Mary College, University of London, London, England, and to the Experiment Station for the Fats and Oils Industry, Milan, Italy, has been completed but final reports have not been received. These grants cover studies on alkaline cleavage of polyunsaturated fatty acids and oxidation of such acids with atmospheric oxygen, respectively.

C. Microbiology and Fermentation

1. Microbial modification of fatty acids. Use of concentrated, non-growing cell suspensions was found to be a more sensitive means than conventional fermentation for detecting microbial modification of fatty acids. Three cultures have been found that modify pelargonic acid and seven that modify oleic acid. Three strains of organisms isolated from soil were found to produce 10-ketostearic acid from up to 20 percent of the 1-percent concentration of oleic acid supplied. The conversion can be consistently accomplished by conventional fermentation techniques.

D. Technology - Process and Product Development

1. Aldehyde oils and derivatives. In engineering research, an improved method for contacting ozone with soybean oil-water emulsions to obtain nearly complete reaction was developed. Products containing 2.25 aldehyde groups per molecule (theoretical maximum 2.45) were obtained. Procedures for conversion of aldehyde oils to acetals in nearly quantitative yields were also developed.

Undesired water-in-oil emulsions encountered in use of this method for ozonolysis could be avoided by using a mixture of equal weights of oil and nonanal, hexanal, or their acetals. These aldehydes, of course, are themselves products of reductive ozonolysis of the oil.

Statistically designed experiments at Archer Daniels Midland Company have identified the significant variables involved in preparation of aldehydes from soybean methyl esters. This contractor has also reported that ozonide formed in the absence of a solvent undergoes rapid, total, and very exothermic decomposition when heated to 120° C.

Contract research at Fabric Research Laboratories has resulted in preparation and characterization of specified linear poly(ester-acetals) and poly(amide-acetals) having molecular weights of 5,000-10,000 and cyclic acetal rings of 5-, 6-, and 7-members. These products are suitable for the studies on crosslinking and adhesion. Hydroxyester and diester acetals derived from methyl azelaaldehydate were successfully converted to interpolymers containing various commercial diesters, glycols, and amines.

2. Cyclic fatty acids. Engineering studies on preparation of cyclic fatty acids are relevant to industrial utilization of soybean oil. Results are reported under Area 12, subheading D-1.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Investigations to Improve Products

Anders, D. E., and Pryde, E. H. 1966. Infrared spectrophotometric procedure for determining azelaaldehydic acid derivatives. J. Am. Oil Chemists' Soc. 43(5), pp. 305-306.

Anders, D. E., Pryde, E. H., and Cowan, J. C. 1965. Amines from aldehydes derived from the ozonization of soybean esters. J. Am. Oil Chemists' Soc. 42(10), pp. 824-827.

Awl, R. A., and Pryde, E. H. 1966. Reduction of methyl oleate ozonolysis products to aldehydes with activated zinc. J. Am. Oil Chemists' Soc. 43(1), pp. 35-37.

Fedeli, E., Valentini, A. F., Lanzani, A., and Jacini, G. (Experiment Station for the Fats and Oils Industry, Milan, Italy). 1965. Ricerche sull'autossidazione delle sostanze grasse polinsature. V. [Research on the autoxidation of polyunsaturated fatty materials. V.] Riv. Ital. Sostanze Grasse 42(10), pp. 488-492.*

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Technology - Process and Product Development

Beal, R. E. 1966. Removal of metal catalysts from aldehyde oils. J. Am. Oil Chemists' Soc. 43(3), pp. 122-124.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

Chemical Composition, Physical Properties and Structure

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Barnes, R. H., Kwong, E., and Fiala, G. 1965. Prevention of coprophagy in the rat and the growth-stimulating effects of methionine, cystine and penicillin when added to diets containing unheated soybeans. J. Nutrition 85(2), pp. 127-131. (N. Y.)

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Technology - Process and Product Development

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AREA NO. 11: SOYBEAN UTILIZATION - FOOD

Problem. Worldwide shortages of dietary protein and of food fats pose a problem that urgently demands solution. Since soybeans can furnish both of these nutritionally essential substances, foreign markets provide a promising outlet for the rapidly increasing production of soybeans in the United States.

U. S. soybeans could play a dominant role in alleviating the protein shortage in developing countries and elsewhere around the world, if soybean meal, flour, protein, and protein concentrates can be successfully used in food products tailored to meet the various nutritional and palatability requirements. Achievement of the maximum share of foreign food markets will require intensive research to acquire more basic information on components that affect nutritional quality, flavor, and other important characteristics of soybean food products. In addition, better knowledge of the effects of processing on these components is needed.

Soybean oil, now the major edible oil of the United States, is the most important source of nutritionally important linoleic acid. However, this oil contains an unstable component (linolenic acid) that limits its use as a liquid oil. To increase opportunities for foreign utilization of soybean oil, more information is needed to show how to eliminate unstable linolenic acid without loss of nutritive value; to determine the extent to which minor constituents influence flavor and other properties of the oil; and to discover methods for modifying hydrogenated soybean oil to achieve desired functional properties such as melting point and texture. A broad program of basic and applied research is required to achieve the objective.

USDA AND COOPERATIVE PROGRAMS

The Department has a continuing, long-range program involving analytical, organic, and physical chemists, biochemists, and chemical engineers engaged in basic and applied research on edible uses of soybean oil, meal, and protein. Food technologists are also required by the program in connection with formulation and organoleptic evaluation of edible products. Objectives of research on edible soybean oil are to identify undesirable flavor components of the oil, to develop basic information on the chemical changes and mechanisms involved in formation or suppression of these components, and to apply the knowledge gained to the development of edible soybean oil having improved oxidative, thermal, and organoleptic stability. Objectives of research on soybean meal and protein are to obtain basic information on the characterization of proteins, enzymes, and other components of soybean meal and to apply the knowledge gained to solution of problems encountered in processing and utilization of soybean meal and protein in food products for foreign consumption.

The Federal scientific effort for research on utilization of soybeans in foods totals 27.1 scientist man-years. Of this number, 6.6 are devoted to chemical composition and physical properties; 16.4 to flavor; .3 to color, texture and other quality factors; .5 to microbiology and toxicology; and 3.3 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition and physical properties (6.6 scientist man-years) includes basic studies on the phenomenon of heat-gelation of alcohol-washed soybean protein and investigation of mass spectroscopy in elucidation of the chemical and molecular structure of glyceride oils and their derivatives. During the reporting period, studies on characterization of components of whey proteins were completed.

Research at Peoria, Illinois, on flavor (14.7 scientist man-years) emphasizes basic and applied studies on selective hydrogenation as a means of stabilizing soybean oil by removal of linolenate. The work includes chemical, physical, and organoleptic evaluation of edible soybean oil products. Other research is devoted to basic studies of the influence of minor constituents of the soybean on the flavor and other edible qualities of soybean protein food products. Research contracts (1.2 scientist man-years*) are in effect at Rutgers, The State University, New Brunswick, New Jersey, for basic studies on heterogeneous catalysts; and at the University of Illinois, Urbana, Illinois, for basic research on homogeneous catalysts. An additional contract at the University of Illinois provides for studies of the mechanism of homogeneous catalysts of hydrogenation by organoleptic complexes. A portion of this effort (.5 scientist man-year) is allocated to research on food uses of soybean oil. During the year, IIT Research Institute, Chicago, Illinois, completed a research contract on development of heterogeneous selective hydrogenation catalysts.

Research on color, texture, and other quality factors involves a research contract (.3 scientist man-year) at the University of Illinois, Urbana, Illinois, for investigation of factors possibly present in soybeans that could cause digestive disturbances.

Research on microbiology and toxicology conducted at Peoria, Illinois, (.5 scientist man-year*) is concerned with a survey to estimate the incidence of aflatoxin in commercial samples of soybeans.

Research at Peoria, Illinois, on technology - process and product development (3.3 scientist man-years) includes engineering studies on production of full-fat soy flour by processes suitable for use in developing countries and on pilot-plant-scale hydrogenation of soybean oil with new selective heterogeneous catalysts. The work on full-fat soy flour is supported by the Agency for International Development and involves cooperation with UNICEF.

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*Work covers more than one commodity; only effort allocated to soybeans is included in total.

The Department also sponsors research on food utilization of soybeans conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition and physical properties involves grants to the Weizmann Institute of Science, Rehovot, Israel, for research on complexes between soybean protein and other components of the meal (5 years, 1961-1966); the University of Tokyo, Tokyo, Japan, for studies on soybean sterols in defatted meal (4 years, 1963-1967); and Kagawa University, Takamatsu, Japan, for investigations of enzymatic hydrolysis of soybean oligosaccharides (3 years, 1966-1969). During the reporting period, research was completed on soybean polysaccharides at the University of Edinburgh, Edinburgh, Scotland; on a chromatographic study of soybean sugars and oligosaccharides at Kagawa University, Takamatsu, Japan; and on soybean saponins at Hebrew University, Rehovot, Israel.

Research on flavor is conducted under grants to the University of Granada, Granada, Spain, for studies on the effect of processing on frying quality of soybean oil (5 years, 1962-1967); Toyo University, Kawagoe, Saitama-ken, Japan, for research on hydrogenation of soybean oil (5 years, 1962-1967); Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on certain metal chelate compounds as catalysts for selective hydrogenation of soybean oil (2 years, 1965-1967); and University of Tokyo, Tokyo, Japan, for investigations on the flavor components of enzymatically or chemically modified soybean meal and proteins (3 years, 1964-1967). During the year, studies on soybean sterols and their effect on stability of the oil were completed at Gdansk Polytechnic, Gdansk, Poland.

Research on color, texture, and other quality factors involves a grant to Sugiyama Chemical Research Institute, Tokyo, Japan, for basic studies on the color reversion of soybean oil (2 years, 1964-1966).

Research on microbiology and toxicology involves grants to the Central Miso Institute, Tokyo, Japan, for studies on miso made from dehulled soybean grits (4 years, 1962-1966); Bar-Ilan University, Ramat Gan, Israel, for studies on miso-type food products for use in Israel (3 years, 1962-1965); Japan Tofu Association, Tokyo, Japan, for studies on the use of U. S. soybeans for making tofu (4 years, 1963-1967); Institute of Chemistry, Academia Sinica, Taipei, Taiwan, for investigation on preparing Chinese cheese from soybeans (5 years, 1963-1968); Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan, for studies on improved strains of Saccharomyces rouxii for making shoyu and miso (5 years, 1963-1968); Japan Shoyu Research Institute, Tokyo, Japan, for comparative evaluation of U. S. and Japanese soybeans and processing methods for making soy sauce (3 years, 1965-1968); and Tokyo University of Education, Tokyo, Japan, for basic studies on development of foods from enzymatically treated soybean protein concentrates (3 years, 1965-1968).

Technology - process and product development involves a grant to the Israel Institute of Technology, Haifa, Israel, for evaluation of the quality of isolated soybean protein for use in Israeli foods (4 years, 1962-1966).

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 4.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Mass spectroscopy of glyceride oils and derivatives. To obtain basic information on the mechanism of catalytic hydrogenation, mass spectroscopic studies were made of the catalytic deuteration of methyl oleate. Results obtained with this labeled system indicated that on platinum catalysts absorption-desorption occurs at about the same rate as hydrogenation, while on palladium absorption-desorption is about five times as fast as hydrogenation. By combination of mass and infrared spectral techniques, procedures were developed for determining not only the number of deuterium atoms in a molecule, but also the number of -CHD- and of $-CD_2-$ groups.

2. Basic studies on soybean protein. "Fingerprint peptide maps" of the four soybean trypsin inhibitors indicated that inhibitors A_1 and B_2 may both be derived from A_2 but that B_1 is definitely different. The inhibitors were also examined by polyacrylamide gel electrophoresis after reduction and reduction-alkylation. Inhibitors A_2 and B_1 behaved differently, whereas A_1 and B_2 resembled each other but differed from A_2 and B_1. Inhibitors A_1, B_1, and B_2 appeared to be heterogeneous. At a pH of 3.75, where no complex between trypsin and soybean trypsin inhibitor exists, trypsin cleaved the inhibitor with formation of another major protein component.

Insolubilization of soybean protein during acid precipitation (pH 4.5) was found to involve both reversible and irreversible reactions. The reversible insolubilization results from disulfide polymer formation and could be reversed with mercaptoethanol. Irreversible insolubilization comprised an initial rapid change involving the 7S fraction followed by a slower decrease in solubility over several hours during which the 2S, 7S, and >15S fractions decreased slowly. Precipitation in the presence of sulfhydryl blocking agents had no effect. On the other hand, dialysis of a water extract of soybean meal against 10 percent sodium chloride and then distilled water resulted in a significant decrease in the amount of insolubilization. This observation suggests that low-molecular-weight components participate in insolubilization. A procedure was devised for quantitative measurement of insolubilization by determination of solubility of acid-precipitated protein in a buffer in the presence and absence of mercaptoethanol. With freshly prepared laboratory samples, only small amounts of protein were solubilized by mercaptoethanol. Commercial samples showed wide variation in the amount of protein solubilized.

3. Minor constituents of soybeans. These studies are being conducted by several foreign institutions under PL 480 grants. Research at the Weizmann

Institute of Science, Rehovot, Israel, has resulted in isolation of three new hemagglutinins. The presence of multiple forms of hemagglutinin and trypsin inhibitor (see item 2, above) strongly suggests proteolytic degradation of parent molecules during protein isolation.

Investigators at Hebrew University, Rehovot, Israel, isolated and characterized a new saponin from soybeans, bringing the total to five known to be present. A comparative study of soybean and alfalfa saponins showed that the two types differ sharply in biological properties, although certain of their components are chemically similar; alfalfa saponins inhibited chick growth, whereas soybean saponins did not. No varietal differences either in saponin content or activity were found in a comparison of six soybean varieties. Although pure saponins isolated from soybeans killed fish by inhibiting cholinesterase (a nerve enzyme) and inhibited food digestive enzymes in certain insect larvae, tests showed that their presence in soybean meal is not detrimental to its nutritive value. In controlled feeding studies no deleterious effect was noted when these saponins were added to diets of chicks or rats in a concentration of five times their normal content in soybean meal. The enzyme-inhibiting effect of the purified saponins was fully counteracted by mixing them with soybean protein, which appeared to complex and thus inactivate them. This project has been completed.

At the University of Tokyo, Tokyo, Japan, soybean sterols have been shown to exist in four different forms: free, ester, glucoside, and acylated glucoside. A chromatographic method for separation of the four forms has been developed.

Studies on soybean polysaccharides were completed at the University of Edinburgh, Edinburgh, Scotland. Results of this research showed that dehulled, defatted soybean meal, which includes food-grade soy flour, contains about 30 percent carbohydrates, of which one-half is oligosaccharides: 8.2% sucrose; 5.5% stachyose; and 1.2% raffinose. The other half is polysaccharides: 8-10% of a neutral arabinogalactan; 5-7% of an acidic polysaccharide complex resembling pectic and tragacanthic acids; and possibly 1% of an arabinan. Soybean hulls have four kinds of polysaccharides: 9-11% galactomannan; 10-12% of the acidic, pectin-type polysaccharide; 9-10% xylan hemicellulose; and 40% cellulose. Knowledge of the polysaccharides opens the way for processing studies directed toward modifying functional and nutritional properties of soybean flours. For example, the acidic polysaccharides offer possibilities for hydrolysis by pectinase to increase solubility of the meal and possibly to increase digestibility.

Studies on sugars and oligosaccharides in soybeans were completed at Kagawa University, Takamatsu, Japan, but the final report has not yet been received. This investigation is being extended under a second grant to the same institution for research on enzymatic hydrolysis of soybean oligosaccharides.

B. Flavor

1. Selective hydrogenation - homogeneous catalysis. Mixtures of chloroplatinic acid and stannous chloride were found to serve as catalysts for the homogeneous hydrogenation of methyl linolenate in methanol-benzene solution. At 65° C. and 220-500 p.s.i., dienes and diene-trienes were the major products. Isomerization was extensive. Further study of the catalytic activity of platinum-tin complexes showed that conjugation was an important initial step preceding reduction. In fact, with the complex $[(C_6H_5)_3P]_2Pt \cdot HSnCl_3$ (I), linolenate underwent more conjugation than reduction to diene. The procedure for producing conjugated oils via iron tricarbonyl complexes (formed by reaction with iron pentacarbonyl) has been improved by the discovery that the complexes can be decomposed with carbon monoxide to yield the conjugated oil and regenerate iron pentacarbonyl for reuse in complex formation. For example, when a soybean oil complex was treated at 180° C. with CO at 3,600 p.s.i., 84 percent of the theoretical yield of iron pentacarbonyl was formed and 82 percent of the polyunsaturates in the oil were conjugated. A study of the ^{13}C NMR spectra of linoleate and butadiene complexes with iron tricarbonyl showed that bonding between iron and the olefinic systems resembles that present in ferrocene.

At the University of Illinois, contract studies on homogeneous hydrogenation showed that methanol was required in the solvent system to achieve selectivity in the use of platinum-tin complexes for hydrogenation of methyl linoleate to monoenes and conjugated dienes. Without methanol, stearate became an important product. These complexes also extensively isomerized methyl oleate if methanol was present. At the University of Illinois, the arsenic- and antimony-containing analogs of I were prepared and compared with I in hydrogenation of soybean esters. Catalytic activity was greatest for the arsenic derivative, whereas conjugation produced was least (64.6 percent) for I and greatest (77.2 percent) for the antimony derivative. Related triphenylphosphine complexes of iron, cobalt, and nickel also were examined. Dibromo-bis-(triphenylphosphine)-nickel catalyzed hydrogenation of methyl linoleate to monoenes. The dichloro derivative showed little activity and addition of stannous chloride decreased both activity and selectivity of the dibromo compound. Di- and trichloro-bis-(triphenylphosphine)-iron both catalyzed reduction of linoleate to stearate only. Dichloro-bis-(triphenylphosphine)-cobalt was inactive.

Under a PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, a novel procedure was developed for preparation of metal chelates by chromatography on adsorbent impregnated with metal salts. These chelates are for use in studies on selective hydrogenation.

2. Selective hydrogenation - heterogeneous catalysis. A laboratory process for preparation of methyl cis-15-octadecenoate was developed. It is based on use of chromatography with a silver-treated macroreticular exchange resin to separate the mixture of 9-, 12-, and 15-octadecenoates isolated by counter current distribution of hydrazine-reduced linoleate. Mathematical theory

and a digital computer program were developed to describe the various modes of operation of counter double current distribution and to provide information needed for determining optimum operating conditions and for interpreting results. A program to generate overlapping distribution functions was written for a general-purpose analog computer. The position, width, amplitude, and skewing of each function can be varied to match experimental data. Component percentages can be calculated from integral curves. Matching is done rapidly by means of an oscilloscope, and computer settings are related to parameters of the distribution. These achievements represent important advances in our capacity to analyze complex reaction mixtures resulting from hydrogenation and to provide meaningful interpretation of the experimental data.

Contract research at IIT Research Institute on preparation of new heterogeneous catalysts has been completed. Study of various metals supported on molecular sieves revealed catalysts that are nonselective for hydrogenation of the 15,16 double bond of methyl linolenate but that are selective for this double bond when linolenate is incorporated into the triglyceride structure of an oil. In general, these catalysts attack linolenate three times more rapidly than linoleate.

3. Evaluation of edible soybean oil products. Soybean oils hydrogenated with commercial copper-barium chromate catalysts were evaluated for quality. Initial flavor scores for all oils treated with citric acid were good. These oils had less oxidative stability than comparable oils hydrogenated with nickel catalysts, but citric acid treatment gave improved stability to both types.

In chromatography of hydrocarbons on activated alumina, procedures have been discovered that achieve one or more of the following improvements: (1) extension of upper limit to hydrocarbons above C_{10}; (2) selective and irreversible adsorption of olefins, including separation of cis,trans isomers; (3) efficient and rapid hydrocarbon resolution; and (4) controlling retention time and column efficiency by appropriate selection of the carrier gas. Accurate determination of hydrocarbons present in soybean oil is important to development of analytical methodology for rapid and objective evaluation of oil quality.

Scientists at the University of Granada, Granada, Spain, have developed an accurate yet simple test for the measurement of fat penetration in food products (error of 0.01 percent based on 2,500 measurements) and have shown that cottonseed oil and olive oil penetrate less than soybean oil, winterized soybean oil, or peanut oil. Results continue to show that acceptance of soybean oil is equal to that of olive oil and that soybean oil is still more acceptable than winterized soybean oil. Study of vessels for frying indicated that with olive oil more acceptable chips were obtained when a glass vessel was used. With soybean oil and winterized soybean oil, glass, aluminum, and steel vessels were equally suitable.

4. Effect of sterols on flavor stability. Research under a PL 480 grant to Gdansk Polytechnic, Gdansk, Poland, has shown that although the bleaching process caused drastic transformation of sterols in soybean oil, neither sterols nor their transformation compounds were important in the initial stages of oil autoxidation and thus had little influence on the development of undesirable flavors and odors in soybean oil. This project has been completed.

5. Flavor components of soybean meal and protein. Lipids extracted from defatted soybean meal were separated into two groups designated "residual oil" and "bound lipids." The flavor components of the defatted meal were found to be present in the bound lipids and in a non-lipid fraction. Phenolic constituents appear to be present in quantities too small to influence flavor characteristics.

At the University of Tokyo, Tokyo, Japan, volatile carbonyl compounds from soybeans and defatted soybean flour have been partially identified. They are similar, in part, to the volatile flavor products from soybean oil. Studies on the enzymatic modification of soybean protein and the flavors derived therefrom have been initiated. This research is being conducted under a PL 480 grant.

C. Color, Texture, and Other Quality Factors

1. Flatulence factor of soybeans. In contract research at the University of Illinois, it was shown that fermentation of soybean oligosaccharides in the ileum and colon is apparently the explanation for flatulence caused by soybeans. Phenolic constituents of the soybean, such as genistin and chlorogenic and ferulic acids, appear to inhibit flatulence. Although sodium soy proteinate and sodium caseinate were effective in suppressing flatulence caused by soy flour, they were ineffective for navy bean flours. Soybean constituents and other special fractions used in this work were provided by the Northern Division.

2. Color reversion of soybean oil. In studies under a PL 480 grant to Sugiyama Chemical Research Institute, Tokyo, Japan, distinct progress on the cause and prevention of color reversion in soybean oil has been made. High-moisture beans have been associated with the cause of color reversion of soybean oil. The red color component which develops in the oil has been identified as tocored. The oxidative or enzymatic mechanism by which tocored develops is under investigation.

D. Microbiology and Toxicology

1. Aflatoxin investigations. Studies on toxins produced by molds are important to utilization of soybeans in foods. Results are reported under Area 3, subheading B-2.

2. U. S. soybeans for making tofu. In studies conducted under a PL 480 grant to the Japan Tofu Association, Tokyo, Japan, 26 varieties and experimental strains of U. S. soybeans were tested for use in making fresh tofu. Hawkeye proved to be the best in both laboratory experiments and pilot-scale trials.

3. Studies on miso and shoyu. These studies are being conducted by several foreign institutions under PL 480 grants.

At Bar-Ilan University, Ramat Gan, Israel, excellent miso-like products have been prepared from defatted soybean meals treated enzymatically prior to fermentation. The time of fermentation needed to make a satisfactory product by this procedure is about 7 weeks, as compared to the 3 to 6 months needed with conventional processing. Application has been made for a U. S. patent covering this development.

Investigators at the Central Miso Institute, Tokyo, Japan, tested 26 varieties and strains of soybeans for their suitability for making miso. Mandarin and Kanrich were judged superior.

Studies to find superior strains of the organism Saccharomyces rouxii for use in making miso and shoyu were continued at the Noda Institute for Scientific Research, Noda-shi, Chiba-ken, Japan. Good fermentative strains were found to exist in both mating types of the organism. However, those strains of the same mating type as NRRL Y-2547 were generally better with regard to flavor-producing abilities than those of the opposite mating type.

4. Chinese cheese (sufu). Chinese cheese is soft and difficult to retain in block shape. However, it can be well preserved for 3 months by heating it in a drying oven and sealing it in a polyvinyl chloride plastic bag which is then coated with paraffin. This work is being conducted under a PL 480 grant to the Institute of Chemistry, Academia Sinica, Taipei, Taiwan.

E. Technology - Process and Product Development

1. Full-fat soybean flour. In research supported by the Agency for International Development, engineers at the Northern Division have developed a simple hand process for producing full-fat soybean flour in villages and rural areas of developing countries. Soybeans are soaked overnight, boiled 10 to 15 minutes, air-dried, cracked, dehulled, and ground. All that this processing requires is an open fire and human muscle. Cracking, dehulling, and grinding are accomplished with simple, inexpensive, commercially available machines designed for manual operation.

Flours made by the hand process compared very favorably with commercial samples. Beverages prepared with the experimental flours showed good dispersion stability for 24 hours or longer. In a study of bacteriological and oxidative stability, samples of full-fat flour prepared by the hand process were stored 40 weeks at room temperature. Microbial counts remained

very low during this period. Taste panel evaluation showed no rancidity or off-flavors. Peroxide value at the end of the test was lower than that of soy flour made by a conventional process. Fifty pounds of the hand-process flour have been sent to Human Nutrition Research Division for evaluation as a component of various foods.

2. Quality of isolated protein for use in Israeli-type foods. Most of the research on the processing factors that affect yield, color, and nutritive value of isolated soy protein has been completed. Research on the use and functionality of isolated protein in foods is expanding and excellent progress has been made. Valuable information of direct application increasing the use of soybean protein products has been obtained. Some of the major U. S. soybean processors have expressed a great deal of interest in this project. These studies are being conducted under a PL 480 grant to the Israel Institute of Technology, Haifa, Israel.

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AREA NO. 12: FLAX UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Traditional markets for linseed oil, the major drying oil produced and used in the United States, are threatened by widespread use of synthetic products derived from nonagricultural sources. In recent years, annual domestic use of linseed oil has ranged from 363 to 394 million pounds in contrast to the postwar high of over 700 million pounds in the early 1950's. This decrease was caused primarily by displacement by synthetic materials capable of better performance, particularly in protective coatings.

To restore the level of use of linseed oil, new or expanded markets are urgently needed. Such markets can be achieved by an adequate program of basic and applied research. Recent studies by Department scientists have resulted in commercial manufacture and sale of linseed emulsion paints for exterior use that are competitive with synthetic resin emulsion paints. Other new products from linseed oil to which Department research is contributing are protective coatings for use in curing fresh concrete and in preventing its deterioration from de-icers and freezing and thawing in winter. These new uses have improved the competitive position of linseed oil in relation to synthetics, but additional research is needed to insure maximum acceptance and consumption of linseed oil in these new markets and to provide still other new or improved products that can maintain and increase its use in all types of protective coatings.

Other new outlets can be realized by chemical modification of linseed oil to obtain materials that will find applications in the multibillion-pound annual market for products of the organic chemical industry. To furnish a sound basis for chemical modification, a broad program of basic research on linseed oil is required to furnish new leads and new concepts that will point the way to those products having the best chance for acceptance.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program involving analytical, organic, and physical chemists and chemical engineers engaged in basic research on the chemical reactions of linseed oil and its component fatty acids and in the application of the knowledge gained to the development of new or improved products for the chemical and protective coating industries.

The Federal scientific effort concerned with research on industrial uses for linseed oil totals 18.2 scientist man-years. Of this number, .7 is devoted to chemical composition, physical properties and structure; 13.7 to chemical and physical investigations to improve products; .7 to microbiology and fermentation; and 4.1 to technology - process and product development.

Research at Peoria, Illinois, on chemical composition, physical properties and structure (.7 scientist man-year) involves study of mass spectroscopy for elucidating the chemical and molecular structure of glyceride oils and their derivatives.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (11.4 scientist man-years) emphasizes basic studies on the chemistry of linseed oil and linseed fatty acids with the objective of discovering new reactions and derivatives having potential applications in the chemical and protective coatings industries. The work also includes basic investigations of problems related to development of emulsion paints and coatings from linseed oil and to durability of linseed oil films. During the reporting period, projects relating to water-soluble vehicles and to new vinyl ester derivatives of linseed oil were completed.

Research at Peoria, Illinois, on microbiology and fermentation (.7 scientist man-year) is concerned with exploration of the possibilities of preparing new and useful derivatives by fermentative modification of fatty acids.

Research contracts (1.3 scientist man-years*) are in effect with North Dakota State University of Agriculture and Applied Science, Fargo, North Dakota, for investigations of aldehyde oils as components of protective coatings; and with Stanford Research Institute, Menlo Park, California, for studies on properties and reactions of new vinyl copolymers of linseed oil. Also included in the total is the Northern Division's share in support of a cooperative agreement among the Division, North Dakota State University, and the National Flaxseed Processors Association. Research under this agreement is conducted at North Dakota State University and involves preparation and evaluation of linseed oil derivatives for use in improving durability of protective coatings.

Research at Peoria, Illinois, on technology - process and product development, involved engineering studies completed during the reporting period on preparation of cyclic fatty acids and aldehyde oils from linseed oil. Research contracts (2.6 scientist man-years*) are in effect with Kansas State University, Manhattan, Kansas, for studies on the use of linseed oil as a single coating for both curing and protection of concrete; with Fabric Research Laboratories, Dedham, Massachusetts, for investigations on poly(ester-acetals) and poly(amide-acetals) derived from aldehyde oils; and with Archer Daniels Midland Company, Minneapolis, Minnesota, for pilot preparation of various aldehyde oil products needed for developmental investigations. During the year, contract studies on protection of air-entrained concrete with linseed oil were completed by Kansas State University.

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*Work covers more than one commodity; only effort allocated to flax is included in total.

The Department also sponsors research in this area under grants of PL 480 funds to foreign institutions. Chemical and physical investigations to improve products are conducted under grants to the Experiment Station for the Fats and Oils Industry, Milan, Italy, for studies on stereospecific polymerization of polyunsaturated fatty esters (2 years, 1965-1967),* and to the Regional Research Laboratory, Hyderabad, India, for exploratory research on hydroxylation reactions of linseed and safflower oils (5 years, 1963-1968). During the reporting period, research* was completed on alkaline cleavage of polyunsaturated fatty acids at Queen Mary College, University of London, London, England; and on oxidation with atmospheric oxygen to obtain new linseed and soybean oil derivatives at the Experiment Station for the Fats and Oils Industry, Milan, Italy.

Research on microbiology and toxicology involves a grant to the University of Baroda, Baroda, India, for studies on production of microbial lipases useful for modifying vegetable oils (5 years, 1965-1970).*

PROGRAM OF STATE EXPERIMENT STATIONS

State stations did not report research in this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Mass spectroscopy. Mass spectroscopic investigations of chemical and molecular structure of glyceride oils and their derivatives are relevant to industrial utilization of linseed oil. Results are reported under Area 11, subheading A-1.

2. Minor constituents of linseed oil. Completion of research under a PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy, was noted last year. The final report, which was subsequently received, indicates that a number of significant results were achieved during the work. The unsaponifiable material from an Italian linseed oil was separated into hydrocarbons, esters, alcohols, and sterols. Most of the components in each of these fractions were isolated and identified. A comparison of the components in the unsaponifiable fraction from Italian linseed oil with six American linseed oils showed that the same classes of compounds were present. Generally those compounds most abundant in the Italian oil were most abundant in American oils. No significant difference could be attributed to geographic origin.

At least 43 saturated hydrocarbons (chain lengths from C_{11} to C_{35}) were found in the unsaponifiable material of linseed oil. Squalene was most

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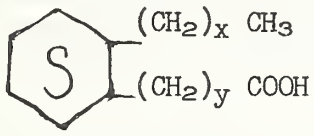
*Effort prorated between linseed and soybean oils.

prevalent among the olefinic-type hydrocarbons. The most abundant straight-chain alcohols were C_{22}, C_{24}, C_{26}, and C_{28}. The major terpene alcohols were cycloarthanol, phytol, geranyl-geraniol, 24-methylenecycloarthanol and an unidentified C_{30} alcohol that gives a positive Fitelson test but is different from butyrospermol, the positive Fitelson substance in tea seed oil. Sterols were found to make up 45 percent of the unsaponifiables and were identified as stigmasterol, β-sitosterol (the most abundant sterol), and campesterol.

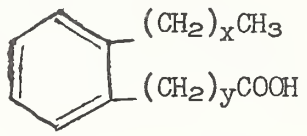
The effect of minor constituents on the spreading and wetting properties of the Italian and six American linseed oils was studied by interfacial tension, surface tension, and contact angle measurements. This area of research proved particularly difficult because these physical methods did not appear sensitive enough to show the small differences encountered. It is also possible that the minor constituents *per se* do not play a major role in the wetting and spreading properties of linseed oil.

B. Chemical and Physical Investigations to Improve Products

1. Cyclic fatty acids. Four of the six anticipated isomers of C_{18} saturated cyclic acids (I) and aromatic acids (II) from linseed oil have been synthesized.



I.



II. (x + y = 10)

The isomers prepared were those for x = 0, 1, 2, and 3. Comparisons with the mixtures obtained by alkaline cyclization of linolenic acid showed that the principal isomer formed is that corresponding to x = 2. This isomer would result from the 10,12,14-conjugated isomer of linolenic acid.

In studies of cycloaddition reactions of olefins, ethylene was found to react with *cis,trans*-conjugated linoleic acid only if an isomerization catalyst (NaOH) was present. Other cycloaddition products were prepared from conjugated methyl linoleate and tetrafluoroethylene, hexafluoropropylene 1,1-dichloro-2,2-difluoroethylene, and chlorotrifluoroethylene. These products were hydrogenated prior to further characterization and study of properties. Adducts were also prepared from even-numbered olefins (C_6-C_{20}) and octadecadienoic acids. Yields were about 20 percent.

Toxicity tests on cyclic alcohols revealed no irritation to the skin or eye of the rabbit. Feeding tests at the 1-percent level in the diet of rats produced growth inhibition that appeared to be due to reduced food intake rather than to direct toxic action.

2. New polymers and derivatives for use in water-soluble and other coatings. Final studies on water-soluble vehicles included examination of film properties of amine-neutralized resins containing linseed fatty acids, tris(hydroxyethyl)aminomethane, and various dibasic acids. Films were deposited from solutions containing approximately equal parts of resin and solvent (isopropanol-water). With the exception of a resin made with fumaric acid, drying times were longer than those of films deposited from toluene solution. The aqueous alcoholic resin solutions wet a steel substrate but the resulting individual films did not have uniform thickness.

A number of new polyesteramide resins having potential as coatings were synthesized. These include products (I) made from linseed dihydroxyamide (10 percent molar excess) and various dibasic acids and products (II) made from tris(hydroxyethyl)aminomethane, linseed fatty acids and various dibasic acids. The products (I) were generally poorer than those made from equimolar amounts of the same reactants, but after modification by reaction of toluene diisocyanate with the excess hydroxyl in the resins, the products gave hard films that exhibited significantly less time lapse between dry-to-touch and tack-free times. The products (II) gave films comparable in water-alkali, and toluene resistance to conventional alkyd resins. Furthermore, the experimental resins dried more rapidly and yielded much harder films. A polyurethane prepared from linseed dihydroxyamide and toluene diisocyanate gave films that dried tack-free in 4 minutes to a hardness of 54 and resisted 5 percent alkali for over 240 hours.

In studies on sulfur-containing products, some substitution was shown to occur in addition of hydrogen sulfide to the double bond of methyl oleate. Hexane 1,6-dithiol was added to linseed oil to give a product containing 1.5 hexanedithiol units per mole of glyceride. This product was slow to dry at room temperature but gave good baked films. These addition reactions were induced at 25° C. by ultraviolet light.

Films prepared from linseed oil reacted with liquid hydrogen sulfide at -70° C. under ultraviolet radiation dried very slowly at room temperature, but films baked 0.5 hour at 275° C. in CO_2 atmosphere had a hardness of 6 and resisted alkali for 30 hours. Curing of this vehicle evidently proceeds by a novel mechanism that does not involve participation of oxygen.

Contract research at Stanford Research Institute showed that in the copolymerization of C_{18} methyl esters with ethyl acrylate, styrene, and acrylonitrile reactivities were in the order: linoleate < linolenate < conjugated linoleate. Conversions of 80-93 percent were obtained in copolymerization of ethyl acrylate with linseed oil and light-bodied linseed oil. Useful copolymers could probably be made from acrylonitrile and any of the C_{18} esters. Practical copolymerizations do not, however, appear feasible with styrene.

Studies on aldehyde oil coatings under the contract at North Dakota State University showed that films with good properties could be obtained by styrenation of hydroxyethyl methacrylate acetals of aldehyde oils. Urethane resins based on soybean dialdehyde oil showed some superiority to corresponding products based on linseed monoaldehyde oil.

3. Linseed oil films and emulsions. Tensile properties of unsupported pigmented and unpigmented linseed oil films were measured. Unpigmented films were much weaker and elongated more than films containing TiO_2 or SnO_2 pigment. When water soaked, these same films retained about two-thirds of their breaking strength at 50 percent R.H. Zinc oxide gave stronger but less extensible films than did the other pigments, but, when water soaked, films containing ZnO retained only about one-fifth of their 50 percent R.H. breaking strength. Unpigmented films and those containing TiO_2 or SnO_2 showed little or no swelling when soaked in water, whereas those containing ZnO swelled 19 percent. The loss of strength coupled with swelling observed for films containing zinc oxide clearly has significant implications in relation to the well-known tendency for linseed house paints containing zinc oxide to blister, crack, and peel under adverse moisture conditions.

In research on emulsion paints, an ionic emulsion paint formulation was developed. Ingredients are nonbodied linseed oil, ZnO, TiO_2, and only 1 percent total concentration of emulsifiers and dispersing agents. The paint is viscosity stable, dries tack-free in 1 hour, passes the water test after 15 minutes, dries to a hard film on glass in 20 hours, and shows no wrinkling, blistering, or other visible change when a thoroughly dry film is soaked in water for 24 hours.

Research to find ways to increase the durability of linseed oil films has been initiated at North Dakota State University under the cooperative agreement among the Northern Division, North Dakota State University, and the National Flaxseed Processors Association. First experiments involved preparation of mercury derivatives of linseed oil containing 0.12 to 44.3 percent mercury. Derivatives containing 34.3 percent or less mercury were stable to heat, ultraviolet light, and boiling water.

4. Glyceride polymers. Last year it was noted that work had been completed under the PL 480 grant to the Experiment Station for the Fats and Oils Industry, Milan, Italy. The final report, which was subsequently received, indicated that in the thermal polymerization of linseed oil between 240-300° C., polymeric acids do not form intramolecularly in monomeric glycerides below 280° C. Polymerization occurs only between fatty acid chains in different glyceride molecules. Below 280° C., linolenic acid is responsible for most of the polymerization; linoleic and oleic acids participate significantly only at higher temperatures.

Intramolecular dimeric and trimeric acids and, perhaps by a different mechanism, dimeric glycerides may be formed simultaneously. No evidence

for trimeric glycerides was found, but tetrameric and higher glycerides were formed under the most severe conditions studied.

The structure of the dimer acids isolated from the thermally polymerized oils could not be deduced. Ozonolysis products indicated that isomerization of the double bonds occurred to give a mixture of dimer acids. A large fraction of these dimer acids appeared to contain a terminal methylene group.

Research on stereospecific polymerization was begun under the second PL 480 grant to this institution. Preliminary polymerization studies were conducted on several fatty polyene esters with a triethyl aluminum-titanium tetrachloride catalyst system. Experiments at 25° and 40-50° gave only low yields of polymer from either conjugated or nonconjugated esters.

5. Hydroxylation of linseed oil. Hydrogenation of epoxide groups without affecting olefinic unsaturation was successfully achieved. The procedure involves use of a Pd-C catalyst and a solvent, such as methanol or ethanol, containing dissolved silver nitrate. The olefinic bonds appear to be protected by π-complex formation. Patent applications covering this process have been filed in India and in the United States. Study of several alternate routes for hydroxylation was continued, but no new significant results were obtained. This research is being conducted under a PL 480 grant to the Regional Research Laboratory, Hyderabad, India.

6. Aldehyde oils and derivatives. Research on preparation of aldehyde oils and derivatives is relevant to industrial utilization of linseed oil. Results are reported under Area 10, subheadings B-1 and B-2.

C. Microbiology and Fermentation

1. Microbial modification of fatty acids. Research to explore possibilities of microbial modification of fatty acids as a means for preparing new and useful derivatives is pertinent to industrial utilization of linseed oil. Results are reported under Area 10, subheading C-1.

D. Technology - Process and Product Development

1. Cyclic fatty acids. Engineering studies on cyclic acids from linseed oil have essentially achieved the objective--development of a practical cyclization process--and have been terminated. Several significant discoveries were made during the engineering research, including the reactivity of ethylene toward conjugated fatty acids, methods for conducting cyclization in aqueous systems, and advantageous ways of separating and purifying reaction products.

2. Linseed oil coatings for concrete. The final results of the contract research at Kansas State University on protection of air-entrained concrete with linseed oil established that air-entrained concrete does need protection against freeze-thaw damage and that properly applied boiled linseed oil

effectively provides such protection. Use of the Northern Division's linseed oil emulsions rather than solutions in kerosene for application of the oil has multiplied the possible outlets for linseed antispalling compounds, since fire and health hazards are substantially eliminated. These emulsions were accepted for use in the Grant Park underground garage in Chicago.

A second contract was undertaken by Kansas State University to evaluate the performance of these emulsions as a combined curing and antispalling agent. Initial results indicated that concrete coated with boiled linseed oil emulsion developed strength comparable to that of concrete moist-cured or protected by polyethylene sheet during curing.

Field tests conducted under the Northern Division's supervision in Washington, D. C. and in Texas also reveal excellent cures and wear resistance in the cured concrete. A new field test of curing has been initiated at Wichita, Kansas, and a further field test of antispalling activity is underway at Manhattan, Kansas.

3. Aldehyde oils and derivatives. Engineering studies on preparation of aldehyde oils and derivatives is relevant to industrial utilization of linseed oil. Results are reported under Area 10, subheading D-1.

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*Research supported by PL 480 funds.

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None.

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*Research supported by PL 480 funds.

AREA NO. 13: NEW CROPS UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Farmers could achieve more economic use of their land if new and profitable crops were available for their choice that would have different end-use patterns from those presently grown. For example, it would be advantageous to develop a new oilseed crop yielding unique fatty acids that could find industrial use in applications for which acids from presently available domestic oilseed crops are unsuitable. To develop a new crop, three basic steps are involved: (1) survey of wild plants, in cooperation with plant scientists, to identify those having both potentially valuable components and promising agronomic potential for use in the United States; (2) detailed physical and chemical studies on components of interest to obtain clues to likely end uses; and (3) selection of the most promising species, followed by additional utilization research to explore uses and demonstrate industrial potential, as well as by additional agronomic research to establish proper cultural practices and to select the best strains and varieties. Only after these steps have been successfully accomplished can a proposed new crop be offered to agriculture and industry for introduction and development. Obviously, a program of this type is a long-range one. Yet, whether the future of agriculture involves conditions of surplus, of greater emphasis on foods and feeds, or of necessity for greater national self-sufficiency, the nation will benefit from availability of optimum, practical crop plants to serve its needs.

Research on new crops has already revealed several promising plant sources of new products that should have valuable industrial uses. These products include water-soluble gums, pulp fibers, and oils containing unique fatty acids such as hydroxy-unsaturated acids, capric acid, epoxidized acids, and unusual long-chain fatty acids.

One new crop, crambe, has a seed oil rich in erucic acid, which is currently obtained from imported rapeseed oil. Crambe has recently achieved commercialization and the outlook for it to become an important crop is encouraging.

To find still other desirable new crops, continued screening and characterization research is needed. Evaluation of the potential of new materials discovered requires further work on their physical and chemical properties and reactions and on processing to obtain maximum recovery from source plants.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a long-range, continuing program of research involving analytical and organic chemists and chemical engineers engaged in examination of uncultivated plants to find unusual and potentially useful

components and in detailed characterization and evaluation studies of selected components that have the greatest industrial potential and that are obtainable from agronomically promising plants. Plants or seeds for this program are obtained by cooperation with Crops Research Division which procures material from domestic and foreign sources by means of collecting trips or from experimental plantings. Materials from abroad are also made available through Crops Research Division PL 480 projects providing for collecting activities by foreign investigators. All seeds and plants are submitted to a broad chemical screening program to identify sources of unusual and potentially useful components such as oils, fibers, and gums. Components of interest from plants rated by Crops Research Division as having a reasonable agronomic potential for the United States are characterized to obtain clues to areas of utilization of probable interest to industry. On the basis of the results, plants having the highest agronomic potential and containing components of greatest potential industrial value are selected for more intensive utilization research.

The Federal scientific effort for research on new crops as sources of industrial products totals 8.0 scientist man-years. Of this number, 6.2 are devoted to chemical composition, physical properties and structure, and 1.8 to technology - process and product development.

Research on chemical composition, physical properties and structure is conducted at Peoria, Illinois, and includes conduct of the program on screening uncultivated plants for new oils, fibers, gums, and other components of potential value to industry; organic chemical characterization of selected components, especially new oils and fatty acids; and studies on properties of new plant fibers.

Research on technology - process and product development, also conducted at Peoria, Illinois, is concerned with studies on pulping new fiber plants and evaluation of the pulp in paper, structural boards, and related products. During the reporting period, studies on fiber plants other than kenaf were completed and emphasis on kenaf was increased accordingly.

The Department also sponsors research in this area conducted by foreign institutions under grants of PL 480 funds. Research on chemical composition, physical properties and structure involves grants to the Institute of General Chemistry, Warsaw, Poland, for determination of glyceride structure of erucic acid oils (5 years, 1962-1967); and to the Swedish Seed Association, Svalof, Sweden, to find new erucic acid oilseeds (5 years, 1963-1968).

PROGRAM OF STATE EXPERIMENT STATIONS

A combined total of 11.0 scientist man-years is devoted to research on industrial and feed uses of new crops.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition, Physical Properties and Structure

1. Screening for new industrial oils. During the year, 898 samples were received, of which 481 were new species (total new species since inception of the program 4,430). Screening analyses were performed on 620 samples and 441 oils were analyzed. Some of the more significant results are as follows.

Two richer sources of the C_{18} allenic acid, 5,6-octadecadienoic (laballenic) acid, have been found in the mint family: Eremostachys speciosa (21.5 percent laballenic acid in the oil) and Phlomis austra-anatolica (20 percent). The allenic acid found in various species of mint should have unique industrial uses. These species are, therefore, being reviewed with Crops Research Division botanists to determine if agronomic potential of any species is adequate to support developmental studies.

Two species of Coriaria (shrubs) yielded oils containing some 60 percent of a new hydroxy conjugated dienoic acid.

Seed oil of a Picris species from Pakistan contained 30 percent crepenynic acid.

A number of new sources of vernolic acid were found. These comprise five new accessions of Euphorbia lagascae from Spain, four new species of Vernonia from Ethiopia, and one species of Schlectendalia from Uruguay. Analyses were performed on 227 samples from four groups of V. anthelmintica grown in Indiana. Three of the groups showed fairly consistent oil (21-29 percent) and vernolic acid (64-74 percent) content, whereas the fourth showed wide compositional variation (16-26 percent oil; 37-76 percent vernolic acid). These studies have enhanced the prospects for eventual successful commercialization of Vernonia because they show that one group of samples from the Indiana trials maintains variability needed for breeding studies. Selections based on our analyses will be made by cooperating agronomists at Purdue University. Discovery of additional sources of vernolic acid broadens the base for agronomic studies.

Under a PL 480 grant to the Swedish Seed Association, Svalof, Sweden, scientists have been studying the composition of Swedish Cruciferae seeds. By comparison of results from plantings in the different environments of Sweden and Turkey, it was shown that genetic factors are more important than climatic ones in controlling erucic acid content of turnip-rape and white mustard. A spectrum of variability in erucic acid content from 36 percent to 55 percent has been found in oil from single seeds of Brassica carinata, an oilseed with good agronomic potential for the United States. No oilseed of the mustard family has yet been found with more than 66-2/3 mole-percent of 22-carbon acids, so this level may be the highest attainable by plant breeding.

2. Screening for new pulp fiber plants. Samples of Sesbania exaltata and Aeschynomene scabra (both members of the bean family) were evaluated as possible sources of cellulosic fibers. The Aeschynomene appeared inferior to kenaf in several respects, but the Sesbania pulp had properties that might make it preferred for selected types of paper.

3. Characterization of seed oils and component fatty acids. In characterization studies, the structures of the following component acids of seed oils from the source indicated were established: 9,10,18-trihydroxystearic acid (Chamaepeuce sp.); 9-hydroxy-trans-10,cis-12-octadecadienoic and 13-hydroxy-cis-9,trans-11-octadecadienoic acids (Xeranthemum annuum); 15-oxo-cis-18-tetracosenoic, 17-oxo-cis-20-hexacosenoic, and 19-oxo-cis-22-octacosenoic acids (Cuspidaria pterocarpa); 13-D-hydroxy-cis-9,trans-11-octadecadienoic acid (coriolic acid; Coriaria nepalensis). The complete structure was established for α-parinaric acid, i.e., cis-9,trans-11,trans-13,cis-15-octadecatetraenoic acid.

Seed oil of Jurinea anatolica contained 41 percent of esters of triterpene alcohols. Esters present are acetates (76 percent), palmitates (16 percent), and myristates (8 percent). Five triterpene alcohols are involved. All were identified. These unusual triterpene esters exemplify a type of product radically different from the glyceride oils commonly described in our reports. Wide-ranging industrial applications for these unusual oils can easily be imagined, but much further work will be needed to determine if practical consequences can reasonably be anticipated from this discovery.

Monoacetotriglycerides in oil of Euonymus verrucosus were shown to be optically active and to consist of S(-)-α-acetotriglycerides. Most of the glycerides of Impatiens edgeworthii are monoacetotriglycerides. The hydrogenated oil was optically active. Discovery of optically active acetotriglyceride oils is a notable scientific "first." Heretofore, no unmodified, naturally occurring glyceride was known in which optical activity was solely due to assymetry of the β-glycerol carbon atom.

The triglyceride structure of oils high in erucic acid content is being studied under a PL 480 grant to the Institute of General Chemistry, Warsaw, Poland. Based on data from gas-liquid chromatography and enzymatic hydrolysis with pancreatic lipase, the mole percentages of the principal triglycerides present in 10 selected cruciferous seed oils have been calculated. The molecular composition of crambe seed oil appears to be simpler than that of the others, since 66 mole-percent of its total oil can be accounted for by only three principal glycerides, in which the primary alcohol groups of glycerine are esterified exclusively with erucic acid and the secondary alcohol group is esterified with oleic, linoleic and linolenic acids, respectively.

B. Technology - Process and Product Development

1. Kenaf for pulp and paper. A detailed comparison was made of the pulping characteristics of green kenaf, at several stages of maturity, grown in northern Florida and central Illinois. Yields of unbleached screened pulps were 55 percent for Florida kenafs harvested at 120 or 150 days, whereas the maximum yield for Illinois kenaf was about 50 percent for frost-killed material. Florida kenaf harvested at 180 days and frost-killed Illinois kenaf gave about the same yield of bleached pulp (47 and 45 percent, respectively). Florida material gave the higher yields of bleached pulp in other comparisons. Differences in performance of paper attributable to geographic location were not great.

Further studies on bleaching chemi-mechanical kenaf pulps resulted in an improved and more economical procedure for achieving a brightness of 60 for newsprint formulations. Newsprint-type papers have been achieved that are equivalent to commercial newsprint except for a slight deficiency in opacity.

Industrial interest in annual plants for pulp remains at a high level among paper companies, which have expressed the belief that annual pulp crops will eventually be used on a large scale. Our most recent information indicates that the increasing scarcity and cost of labor is the motive force in this direction, not shortage of wood pulp. Both the high yield and annual harvest favor a crop such as kenaf. For example, an acre of loblolly pine produces, at 15-year intervals, about 2.5 tons of material at 50 percent moisture, including bark. Sustained annual yields of kenaf solids run from 5 to 7 tons (dry basis) per acre. The possibility of mechanized handling of an annual plant grown on convenient and relatively compact acreage is unquestionably attractive to industry. Kenaf may well prove to be an annual crop suitable for the type of operation now foreseen by industry.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

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Technology - Process and Product Development

Miller, D. L. 1965. Kenaf--A potential papermaking raw material. Tappi 48(8), pp. 455-459.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

See next area.

AREA NO. 14: NEW CROPS UTILIZATION - FEEDS

Problem. The research program on new crops is a long-range effort to find new and profitable crops that would have different end-use patterns from those now grown. By providing a wider range of choices, the availability of new crops should enable farmers to achieve more economic use of their land. A more extended discussion of the problems involved in finding and developing a new crop is given under Area 13, New Crops Utilization - Industrial Products.

One of the most promising approaches is to search for plants whose seed oils contain potentially useful fatty acids that either are not now available commercially or must be obtained from foreign sources. However, for a new oilseed crop to achieve maximum utility and economic value, it is desirable to obtain, as a byproduct, a palatable and nutritious meal suitable for animal feeds. Thorough investigation is needed, therefore, to determine the probable utility of new oilseed meals as feeds; to discover the presence of possibly undesirable minor constituents; and to evaluate the prospects for successful processing of the oilseed to oil and acceptable meal.

It is possible that a wild plant, although not a potential oilseed crop, might in itself be an advantageous new source of protein or other nutritionally desirable substance. As a part of the broad program on screening and characterization of new plants for potentially valuable components, appropriate effort is required to insure that such a possibility will not be overlooked.

USDA AND COOPERATIVE PROGRAMS

The Department maintains a continuing but limited program involving one professional analytical chemist who devotes a portion of his time to screening uncultivated plants to find possible sources of new amino acids and proteins and to study of amino acids and proteins of meals obtained from new potential oilseed crops.

The Federal program at Peoria, Illinois, totals .4 scientist man-year, all of which is devoted to chemical composition and physical properties.

PROGRAM OF STATE EXPERIMENT STATIONS

A combined total of 11.0 scientist man-years is devoted to research on industrial and feed uses of new crops.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Amino acid analyses were run on seed proteins from 32 species representing 7 plant families. This work is part of a comprehensive survey of seeds as protein sources. Results of the survey, which covered 4,000 species, showed that some species contain amino acids in proportions implying good nutritional balance. Although not themselves balanced, proteins of other species were rich in amino acids that could supplement diets based primarily on cereal grains. The data provide a sound basis for agronomic studies, field trials, and animal tests leading to possible new feed and food crops.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

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AREA NO. 15: CRAMBE UTILIZATION
INDUSTRIAL PRODUCTS

Problem. Crambe, a new oilseed crop commercialized in 1965, is the first plant included in the research program on new crops to achieve this status. Crambe seed oil is rich in erucic acid. Several industrial uses already existed for erucic acid as well as for imported rape seed oil, which formerly was the only source of this acid. However, the greatest impetus to commercialization of crambe was perhaps the discovery that crambe oil performed better than any other known material as a lubricant in continuous casting of steel. This situation emphasizes the importance of finding the most advantageous specific applications that can contribute to utilization of any new crop in its own right.

To insure optimum development of crambe as a new economic crop, possible markets for crambe oil and erucic acid must be explored and those with the greatest industrial potential must be identified and made effective. This goal can be reached through a program of basic and applied research that will provide more information on the chemical and physical properties of crambe oil, its component fatty acids, and their chemical derivatives. When promising leads to possible industrial applications are found, product and process development research will be needed to evaluate the potential and to provide facts and data essential for commercial adoption of crambe oil products in new end uses.

USDA AND COOPERATIVE PROGRAMS

The Department conducts a continuing, long-range program of research involving analytical and organic chemists and chemical engineers engaged in basic and applied research on industrial utilization of crambe oil. The objectives of the work are to obtain new information on reactions of crambe oil and its component fatty acids and to use this information to develop new products for use by the chemical and other industries.

The Federal scientific effort for research on industrial utilization of crambe totals 5.2 scientist man-years. Of this number, 4.0 are devoted to chemical and physical investigations to improve products and 1.2 to technology - process and product development.

Research at Peoria, Illinois, on chemical and physical investigations to improve products (4.0 scientist man-years) is concerned with chemical modification of crambe oil and its component fatty acids, especially erucic acid, to obtain chemical intermediates or derivatives having desirable properties for industrial use.

Research on technology - process and product development involves a research contract (1.2 scientist man-years) with Southern Research Institute, Birmingham, Alabama, for studies on preparation and evaluation of polyamide resins derived from crambe oil.

PROGRAM OF STATE EXPERIMENT STATIONS

A combined total of 1.0 scientist man-year is devoted to research on industrial and feed uses of crambe.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical and Physical Investigations to Improve Products

1. Chemical derivatives from crambe oil. Homopolymerization of vinyl 2-methylpentyl brassylate (VMB) and its copolymerization with vinyl chloride were successfully carried out. Conversions were 75 percent or greater. The results showed that VMB acted as an internal plasticizer for polyvinyl chloride (PVC). A copolymer containing 30 percent VMB had greatest flexibility at low temperatures. Evaluation (conducted at the Eastern Division) of bis-(cyclohexylmethyl) brassylate showed that it had plasticizing properties for PVC intermediate to that of cyclohexyl brassylate and mixed brassylates of cyclohexyl and 2-ethylhexyl alcohols.

Under an informal agreement, Emery Industries, Inc., Cincinnati, Ohio, ozonized crambe free fatty acids and sent 317 pounds of the dibasic acid product to the Northern Division. It contained 31 percent azelaic, 47 percent brassylic, 15 percent other dibasic, and 7 percent monobasic acids.

A series of 12 mixed diesters of mixed dibasic acids (azelaic and brassylic acids from ozonization of crambe acids) was prepared for further plasticizer evaluation at the Eastern Division. In evaluations at the Southern Division, a series of disubstituted amides of erucic acid and mixed crambe acids were found to be more efficient plasticizers for PVC than dioctyl phthalate. The amides from mixed crambe acids displayed more restricted compatibility in comparison to the corresponding erucic acid derivatives.

An improved laboratory method for ozonolysis of erucic acid, in which reaction products are converted to methyl esters, resulted in recovery of 95 percent pure dimethyl brassylate in 88-percent yields. The best previous recovery based on distillation and crystallization of free brassylic acid was 70 percent of theory.

Several reaction products of brassylic acid and ethylene oxide were equal to commercial surfactants used in emulsion polymerization of vinyl chloride.

B. Technology - Process and Product Development

1. Polyamide resins from erucic acid. In contract research at Southern Research Institute, all synthetic steps from erucic acid to nylon-1313 have been placed on a satisfactory preparative basis. Overall yield is 51 percent of theory. Several samples of nylon-1313 were prepared with molecular weights ranging from 12,000 to 47,000. Southern Research Institute has successfully prepared 10 pounds of nylon-1313 for evaluation. Preliminary tests showed that nylon-1313 can be either extrusion or compression molded; that it can be melt-spun into monofilaments and cold drawn; and that it has very low moisture absorption. A procedure was devised for facile conversion of brassylic dinitrile, a principal intermediate in preparing nylon-1313, to the nylon 13 monomer, 13-aminotridecanoic acid.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical and Physical Investigations to Improve Products

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RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

See next area.

AREA NO. 16: CRAMBE UTILIZATION - FEEDS

Problem. The economic value to the farmer and to industry of any oilseed crop is much greater if the meal left after extraction of the oil can be utilized as a palatable and nutritious feed for animals. Crambe, a new and only recently commercialized oilseed crop developed under the new crops research program, yields a meal that, on the basis of amino acid analysis, should be an excellent feed product. However, as is true for other oilseed meals, such as soybean meal, suitable processing is needed to realize fully the anticipated nutritional qualities and to insure maximum acceptability to different types of animals. Needed research includes isolation and characterization of components of crambe meal that are important to nutritional value, flavor, and other essential qualities of a feed. The fate of these components during processing must be investigated in order to learn how to preserve desired components and eliminate or minimize the effects of deleterious ones. Finally, engineering studies are required to translate laboratory findings into economical and practical processes for industrial use.

USDA AND COOPERATIVE PROGRAMS

The Department maintains a continuing, long-range program of basic and applied research involving analytical and organic chemists and chemical engineers engaged in study of the components of crambe meal and in development of effective processes for converting crambe seed to oil and palatable, nutritious meal for animal feed.

The Federal scientific effort for research on feed uses of crambe totals 7.1 scientist man-years. Of this number, 5.3 are devoted to chemical composition and physical properties and 1.8 to technology - process and product development.

Research on chemical composition and physical properties is conducted at Peoria, Illinois, and is concerned with studies on components of crambe meal such as enzymes, other nitrogenous components, pigments, flavor principles, etc.

Research on technology - process and product development, also conducted at Peoria, Illinois, is devoted to engineering studies on processing crambe seed to oil and palatable, nutritious meal.

PROGRAM OF STATE EXPERIMENT STATIONS

A combined total of 1.0 scientist man-year is devoted to research on industrial and feed uses of crambe.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Chemical Composition and Physical Properties

1. Crambe enzymes. Two types of crambe enzyme were isolated. One fraction (insoluble) converts the thioglucoside epi-progoitrin (EPG) to (R)-goitrin; the other (soluble) converts EPG to nitriles. The soluble enzyme fraction, after dialysis, converted EPG to goitrin, and the insoluble fraction at pH 5-6 in the presence of 0.01 M ferrous ion produced nitriles. Addition of EDTA did not prevent nitrile formation in autolysis of whole meal, but added mercaptoethanol, added ferrous ion, or exclusion of oxygen suppressed goitrin formation.

2. Conversion products from epi-progoitrin (EPG). Further study of EPG degradation products formed upon autolysis of crambe meal established the presence of two previously unobserved products in addition to (R)-goitrin and 1-cyano-2-hydroxy-3-butene. These were identified as episulfides, specifically the isomeric 3,4-epithio-1-cyano-2(S)-hydroxy-butanes differing only in configuration at C-3. Chemical treatments of the disilver salt of EPG produced (R)-goitrin and 1-cyano-2-hydroxy-3-butene.

3. Toxicity tests and feeding studies. Toxic properties of epi-progoitrin were demonstrated by feeding studies with rats. Growth was almost normal but pathological changes in the liver and thyroid occurred. Tests with rats and mice showed that the crude "cyano" fraction from autolyzed whole meal had greater toxicity than synthetic 1-cyano-2-hydroxy-3-butene. Purified sinapine bisulfate had no effect on growth rate or feed consumption by rats. Detoxified crambe meals were prepared by autolysis and extraction with aqueous acetone. When fed as the sole protein source to rats, they had protein efficiency ratios of 2.55 and 2.75 (casein, 2.50; soy meal, 2.15). Steaming one of these crambe meals reduced the ratio to 1.51. These studies were conducted with the cooperation of the Pharmacology Laboratory at the Western Division.

B. Technology - Process and Product Development

1. Processing crambe to oil and meal. Sodium carbonate (soda ash) and sodium hydroxide were found to be promising additives for detoxification of crambe meal. Use of 2 percent soda ash with full-fat or defatted crambe meal combined with a moist-steam cooking operation proved superior to ammoniation with respect to apparent detoxification and simplicity of operation.

Comparative palatability of crambe meals prepared at the Northern Division and treated with soda ash, sodium hydroxide, and ammonia was determined in cattle feeding trials at the University of Nebraska. Best palatability, nearly equal to that of soybean meal, was observed for meals treated with soda ash. Ammonia-treated meals also gave good results.

During November 1965, Pacific Vegetable Oil Company--with cooperation and supervision by two Northern Division engineers--processed 740,000 pounds of crambe seed (without dehulling), using the Northern Division soda ash process as closely as equipment permitted. Several sets of conditions were evaluated while the plant was on-stream to determine optimum conditions for processing the larger part of the run. The desolventized meal after extraction contained less than 1 percent oil and about 29 percent protein. Several tons of meal, including a quantity not treated with soda ash, were provided to the Northern Division for use in feeding trials with cattle and other animals. The remainder of the meal and all of the oil were disposed of by PVO through their normal sales channels.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

Chemical Composition and Physical Properties

Daxenbichler, M. E., VanEtten, C. H., and Wolff, I. A. 1966. (S)- and (R)-1-cyano-2-hydroxy-3-butene from myrosinase hydrolysis of epi-progoitrin and progoitrin. Biochemistry 5(2), pp. 692-697.

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Technology - Process and Product Development

Kirk, L. D., Mustakas, G. C., and Griffin, E. L., Jr. 1966. Crambe seed processing: Filtration-extraction on a bench scale. J. Am. Oil Chemists' Soc. 43(5), pp. 334-336.

McGhee, J. E., Kirk, L. D., and Mustakas, G. C. 1965. Methods for determining thioglucosides in Crambe abyssinica. J. Am. Oil Chemists' Soc. 42(10), pp. 889-891.

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RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

(Publications on industrial and feed utilization of crambe)

Chemical Composition and Physical Properties

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AREA NO. 17: FORAGE UTILIZATION - FEED (NORTHERN REGION)

Problem. Tall fescue grass is grown extensively in the Southeast, in the Intermountain States, and in the Pacific Northwest as a forage crop for cattle and other domestic animals. It has excellent agronomic characteristics, producing well on marginal land and remaining green during cool weather when other grasses are dormant. The quality of staying green in the winter is a prime factor in its acceptance. Thirty-five to fifty million acres of fescue are grown for forage use in the Southeastern part of the United States alone.

Cattle grazing on pasture that is predominately tall fescue sometimes develop a disease known as "fescue foot." In severe attacks the animal first becomes lame. The peripheral portion of one or more limbs then develops necrosis, and sloughing of the hooves may occur. Occasionally the tail and ears may be affected. Animals become emaciated and frequently die. The disorder is more apt to occur during cool weather than during the summer months. However, even when conditions are not such as to produce the more dramatic symptoms, cattle sometimes perform poorly on fescue forage, a result which may be attributed to subclinical toxicity.

Pastures may become toxic after several years of freedom from toxicity. Serious outbreaks of fescue toxicity occurred during the winter 1963-64 in parts of Kentucky, Illinois, Missouri, Kansas, and Arkansas. Thousands of head were involved, with morbidity ranging from 1 percent to 99 percent of the herds. For example, 42 of 72 head of cattle became lame after 8 days on one pasture in Missouri. In these outbreaks, the toxic pastures were soil bank lands having long grass that was pastured after the advent of cold weather.

Research to determine the cause of toxicity in fescue and to identify the toxic substance(s) is needed as a basic step in developing a solution to the problem of toxic fescue.

USDA AND COOPERATIVE PROGRAMS

At the Northern Division, Peoria, Illinois, the Department has a program of limited scope that involves one organic chemist engaged in research to isolate and identify the toxic component(s) of tall fescue grass responsible for a cattle disease known as "fescue foot." This research is cooperative with the Kentucky State Experiment Station, which furnishes toxic and non-toxic fescue grass for chemical study and conducts bioassays of fractions and components isolated from fescue at the Northern Division. Liaison is maintained with the fescue breeding program of the Field Crops Research Branch, ARS, through the Agronomy Department of the University of Kentucky and with the Department's Pharmacology Laboratory at the Western Division.

The major part of the Department's research program on forages is maintained at the Western Utilization Research and Development Division, Albany, California.

The Federal program at Peoria, Illinois, totals 1.3 scientist man-years, all of which is devoted to microbiology and toxicology.

PROGRAM OF STATE EXPERIMENT STATIONS

A total of 10.0 scientist man-years is devoted to this area of research.

PROGRESS -- USDA AND COOPERATIVE PROGRAMS

A. Microbiology and Toxicology

1. Fescue toxicity. For the first time, toxicity to the bovine has been demonstrated for a metabolite of a mold isolated from toxic fescue and cultured on non-toxic fescue. Fusarium nivale was grown on non-toxic hay enriched with glucose and peptone. The concentrated 80-percent alcohol extract from this culture gave a positive result in a small animal assay and was toxic to the cow. A crystalline compound of the butenolide class has been isolated from the extracts of F. nivale cultures and found to give a positive test in the small animal assay. The structure of the compound has been determined. Isolation of this irritant, belonging to a class noted for physiological activity, from a mold originally found on fescue, justifies further intensive study even though this compound per se may not be the cause of fescue foot. The work involved cooperation with the Western Division and the Kentucky Agricultural Experiment Station.

PUBLICATIONS -- USDA AND COOPERATIVE PROGRAMS

None.

RELATED PUBLICATIONS OF STATE EXPERIMENT STATIONS

Chemical Composition, Physical Properties and Structure

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Guthrie, W. R., and Collins, E. B. 1965. Factors affecting sorption isotherms of alfalfa. W. Va. Agr. Exp. Sta. Bull. 514T, pp. 1-32. (W.Va.)

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Technology - Process and Product Development

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Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1	Corn, wheat, and other cereal crop utilization investigations--Northern region.			
N1 1-58 (Rev. 2)	Operation and improvement of a culture collection of molds, yeasts, bacteria, and actinomycetes to provide a reservoir of authentic microorganisms for use in making antibiotics, vitamins, chemicals, polymers, assays, and identifications of importance to the national welfare.	Peoria, Ill.	Yes	1-C-1
N1 1-175	Investigations on the carotenoid pigments of wet- and dry-milled fractions from corn, including high-amylose types, and of yellow-endosperm sorghum to provide data basic to the most effective utilization of industrial products and fractions from corn and sorghum in feeds.*	Peoria, Ill.	Yes	3-A-2
N1 1-178	Investigations on the molecular size and state of aggregation of the amylose and amylopectin components of high-amylose corn starches to provide information basic to industrial utilization.*	Peoria, Ill.	Yes	1-A-3
N1 1-179	Basic studies on the chemical structure of the amylose and amylopectin components of high-amylose corn starches to provide information needed for effective industrial utilization of these new starches.*	Peoria, Ill.	No	
N1 1-181	Studies of the effects of conditioning treatments of wheat on morphological and histochemical characteristics of milled fractions to provide information basic to the production of industrially useful fractions from wheat.*	Peoria, Ill.	Yes	5-A-1
N1 1-182 (Rev.)	Studies on protosexual yeasts, their existence, characteristics, and phylogenetic relationships as a basis for developing new yeasts and new processes for the fermentative conversion of cereal grains to new products.	Peoria, Ill.	Yes	1-C-2
N1 1-184	Chemical conversion of wheat flour into a variety of hydrophilic polymers having a wide range of solubilities and viscosities in aqueous dispersions to meet specific industrial requirements for sizes, adhesives, and thickeners.*	Peoria, Ill.	Yes	4-B-3
N1 1-187	Isolation and characterization of physiologically active nonprotein nitrogenous substances in corn and corn-milling products as a basis for applied processing studies to increase the use of corn.*	Peoria, Ill.	Yes	3-A-1
N1 1-190(C)	Investigations on methods for the chemical preparation and characterization of amino derivatives of cereal starches by replacement of nonglycosidic hydroxyl groups to obtain new starch products having increased stability to water, dilute acids, and alkali.*	Columbus, Ohio	Yes	1-B-3
N1 1-194	Search for microorganisms and a fermentative process to convert cereal grain products to xanthophylls that induce desirable pigmentation of poultry products when added to feed.*	Peoria, Ill.	Yes	3-B-1
N1 1-195(C)	Investigations on the alkaline desulfurization of wheat gluten proteins to provide a basis for developing improved modifications of wheat products having utilization potential.	Lafayette, Ind.	Yes	4-A-2

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-196	Chemical investigations on amylomaize selections to guide corn breeders in the development of commercial hybrids containing high-amylose starch for industrial use.	Peoria, Ill.	Yes	1-A-1
N1 1-200	Evaluation of modified cereal flours and starches as sizing agents, coating adhesives, and wet-end additives for paper in large-scale, high-speed continuous runs.*	Peoria, Ill.	Yes	1-D-1
N1 1-203(C)	Investigations on the control of a selected complex reaction of starch or related carbohydrates through controlled fluid flow dynamics and reaction conditions to provide a basis for process design and improvement leading to increased utilization of cereal grains.	Baltimore, Md.	Yes	1-B-5
N1 1-204	Chemical investigations on the molecular structure of the protein, glutenin, present in wheat gluten as a basis for increased industrial utilization of this raw material.	Peoria, Ill.	Yes	4-A-1
N1 1-205(C)	Investigations on reactions of difunctional mercaptans with dextrose, starch, or related carbohydrates to form polymers having potential industrial value.*	Tucson, Ariz.	Yes	1-B-2
N1 1-208	Investigations on the conversion of cereal grains to economical and efficient soluble fermentation substrates through the action of microbial enzymes, as a basis for increasing the use of these grains by the fermentation industry.*	Peoria, Ill.	Yes	1-C-3
N1 1-209	Investigations on the applicability and evaluation of chemically modified cereal grain flours and fractions as ingredients, agents, and adhesives in pulp and paperboard products as a basis for increasing industrial use of cereal grains.	Peoria, Ill.	Yes	1-D-1
N1 1-210	Investigations on the preparation of water-dispersible hetero-derivatives of starch to obtain products having a wide range of properties for the production of adhesives, sizings, and other additives for applications in paper and related industries.	Peoria, Ill.	Yes	1-B-7
N1 1-211	Chemical reaction studies on wheat gluten and its component proteins seeking methods of modification to give properties better suited for industrial uses.*	Peoria, Ill.	Yes	4-B-1
N1 1-212	Investigations on the production of low-density plastic foams from starch-derived glucosides and related starch derivatives as a basis for increasing the industrial utilization of cereal starches.	Peoria, Ill.	Yes	1-B-6
N1 1-213	Pilot-plant investigations on wheat dry-milling and fractionation methods for producing a wide variety of products for use in foods, feeds, and industrial products.	Peoria, Ill.	Yes	5-C-1
N1 1-214(C)	Engineering studies on the application of pneumatic fluidization to the reactions of wheat flour with hydrogen chloride as a basis for producing sizing agents for paper.	Ames, Iowa	Yes	4-D-2
N1 1-215	Investigations of the reaction of dialdehyde starch with casein, soybean protein, soy flour and dried animal blood for the production of improved wood adhesives.	Peoria, Ill.	Yes	1-B-7

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-216	Pilot-plant investigations of dry-milling operations to obtain increased yields of prime goods and oil from old and artificially dried corn and to develop a prototype corn degerminator having improved corn degerminating characteristics for production of higher quality dry-milled products.*	Peoria, Ill.	Yes	2-C-1
N1 1-217	Investigation of methods for producing microbial polysaccharides from cereal grains by continuous fermentation to reduce production costs allowing increased utilization of these potentially useful gums.	Peoria, Ill.	Yes	1-C-5
N1 1-218(C)	Stabilization of vegetative cells of Bacillus popilliae grown on cereal-based media for use as an infecting agent against the Japanese beetle.	Manhattan, Kans.	Yes	1-C-4
N1 1-219(C)	Study of role of enzymes and enzyme activity in the formation of spores of Bacillus popilliae and Bacillus lentimorbus as a basis for the mass production of biological insecticides by fermenting cereal grain.	East Lansing, Mich.	Yes	1-C-4
N1 1-220(C)	The transfer of genetic determinants of sporulation from one microorganism to another, as a basis for applied studies on the fermentative production of spore dusts for the control of Japanese beetle infestations.	Minneapolis, Minn.	No	
N1 1-221(C)	Study of the sporulation factor produced by bacilli and its possible use in Bacillus popilliae and Bacillus lentimorbus to develop a fermentation process for the production of spore dust to control Japanese beetle infestations.*	Urbana, Ill.	No	
N1 1-222(C)	Studies on the mechanism and kinetics of radiation and ceric ion induced grafting of cereal starches with vinyl-type monomers previously shown in exploratory studies to graft readily and efficiently with promise for new industrial outlets for starch.	Menlo Park, Calif.	Yes	1-B-4
N1 1-223(C)	Development studies on the semi-pilot-plant scale production of cereal grain xanthides and their use and evaluation in making corrugating board and linerboard for corrugated boxes.	Columbus, Ohio	Yes	1-D-1
N1 1-224	Development of methods and processes to reduce viable microorganisms in wheat flour as it is produced in the mill.	Peoria, Ill.	Yes	5-B-1
N1 1-225	Investigations on the development of new fermented wheat foods through the use of Oriental-type food molds as a basis for increasing export markets for U. S. wheats.	Peoria, Ill.	Yes	5-B-2
N1 1-226	Investigations on formation and properties of amino and peptide derivatives of starch to provide a basis for the development of industrially useful products from cereal grains.*	Peoria, Ill.	Yes	1-B-3
N1 1-227	Investigations on the conversion of cereal xanthates to xanthides in physical forms suitable for use in papermaking.	Peoria, Ill.	Yes	1-D-1
N1 1-228(C)	Investigations on the interaction of "V" amylose with small molecules to provide basic information on the helical structure of amylose from high-amylose corn starch.*	Tempe, Ariz.	Yes	1-A-3

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-229	Investigations on processing methods for wheat to minimize radioactive contamination in milling products.	Peoria, Ill.	Yes	5-C-2
N1 1-230(C)	Investigations on the synthesis of terminal C4-modified maltooligosaccharides for use in studying enzyme modifications of cereal starches.	Carbondale, Ill.	No	
N1 1-231(C)	Stabilization of beta-carotene in dried mycelium and in extracted form as a contribution to commercialization of beta-carotene produced by fermentation of cereal grain.	Cambridge, Mass.	Yes	3-B-1
N1 1-232(C)	Investigations on the vinylation of methyl glucoside by reaction with acetylene and on the properties and reactions of the products as a basis for development of new industrial outlets for cereal grains.	Tucson, Ariz.	No	
N1 1-233(Gr)	Studies on the types and variations of starch granules within the endosperm of genetically different high-amylose corns to provide fundamental information important to the processing and utilization of high-amylose corn.	Lincoln, Nebr.	Yes	1-A-2
N1 1-234(Gr)	Investigations of two-phase submerged fermentation processes as means for increasing yields and/or concentrations of products obtained by fermentation of cereal grains.	Ithaca, N. Y.	Yes	1-C-5
N1 1-235(C)	Investigation of the morphological changes involved in the transition of Bacillus popilliae from vegetative cells to spores for controlling Japanese beetle infestations.	Houston, Tex.	Yes	1-C-4
N1 1-236(C)	Investigation on the isolation and characterization of phenolic pigments of grain sorghum to provide basic information related to the discoloration of milled sorghum and its starch.	Bloomington, Ind.	Yes	7-A-1
N1 1-237	Investigation of the characteristics and classification of microorganisms of the section Dubiorugorhizopus of the genus Rhizopus of the family Mucoraceae, as tools for use in the development of fermentations utilizing cereal crops.	Peoria, Ill.	Yes	1-C-2
N1 1-238(C)	Studies on kernel properties and milling and fractionation characteristics of wheats exhibiting a range of kernel hardness and protein content to provide information basic to the production of a range of products for industrial uses and application in baking.	Lincoln, Nebr.	Yes	5-A-2
N1 1-239(Gr)	Basic investigations on the chemical and molecular structure of amyloglucosidases with emphasis on relationship to enzyme formation and action to provide information applicable to the production and use of these enzymes in the utilization of cereal grains.	Lincoln, Nebr.	Yes	1-C-3
N1 1-240	New microbial polysaccharides of commercial value produced from cereal grains: Characterization and structural analysis of previously selected polysaccharides and screening for additional polysaccharides with new and broader range of applicability.	Peoria, Ill.	Yes	1-C-5
N1 1-241	Investigations on molecular structure, aggregation, and interactions of wheat gluten proteins and their chemical modifications to provide basic information related to industrial utilization of wheat.	Peoria, Ill.	Yes	4-A-1

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-242	Chemical transformations of maltose and dextrose to determine differences in reactivity and to produce new compounds of possible industrial use from these cereal starch-derived sugars.	Peoria, Ill.	Yes	1-B-1
N1 1-243	Modification of fermentations by transfer of genetic material in microorganisms.	Peoria, Ill.	Yes	1-C-7
N1 1-244	Exploratory studies on the chemical and physical modification of high-amylose corn starches to improve their applications as coatings and sizings for industrial use.	Peoria, Ill.	Yes	1-B-8
N1 1-245(C)	Development of optimal papermaking processes using cereal grain xanthides made from starch, ground whole grain, flour, bran, shorts, and other dry-milled grain products in blends with wood pulp to produce linerboard, corrugating media, and bag papers.	Columbus, Ohio	Yes	4-D-3
N1 1-247	Exploratory studies on the chemical synthesis and characterization of crosslinked starch derivatives having potential value as paper additives for improvement of tear, stretch, and moisture stability of paper products and for upgrading properties of boxwood, insulating board, and other structural materials.	Peoria, Ill.	Yes	1-B-7
N1 1-248(C)	Preparation and evaluation of selected starch graft copolymers for industrial use in plastic products and industrial coatings.	Menlo Park, Calif.	Yes	1-D-3
N1 1-249(C)	Investigations on the preparation of plastic foam from selected starch polyol derivatives and their evaluation in industrial applications.*	Minneapolis, Minn.	Yes	1-D-2
N1 1-250(Gr)	The reaction of vinyl ethers with carbohydrates, especially D-glucose and starch.	Columbus, Ohio	Yes	1-B-3
N1 1-251(Gr)	Basic studies on the relation of viscoelastic properties of amylose sheets and films to structure and function of added plasticizers.	Princeton, N. J.	Yes	1-A-4
N1 1-252(Gr)	Basic investigations on the organic chemistry of unsaturated and sulfur-containing carbohydrates to provide a basis for the development of new reactions and derivatives of cereal grain starches and related sugars.	Columbus, Ohio	Yes	1-B-3
N1 1-253 (Rev.)	Studies on the production of mycotoxins by Aspergillus flavus and related molds to provide basic information for processing grain into feeds.	Peoria, Ill.	Yes	3-B-2
N1 1-254(C)	Development of improved methods for preserving microorganisms that cannot be satisfactorily lyophilized for use in the fermentative conversion of cereal grain into industrial products.	Rockville, Md.	Yes	1-C-1
N1 1-255	Investigation of the sporulation of milky disease bacteria in vivo and in vitro as a basis for the development of a fermentation process for the production of a pesticidal agent against the Japanese beetle.	Peoria, Ill.	Yes	1-C-4
N1 1-256	Exploratory studies on the preparation of new and novel products from unmodified cereal starches and thin-boiling starches by graft copolymerization with selected vinyl and acrylic monomers.	Peoria, Ill.	Yes	1-B-4

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N1 1-257	Basic studies on the relation of film properties of amylose to molecular organization and structure in order to provide information needed in improving the preparation and properties of films and coatings from amylose and high-amylose starch.	Peoria, Ill.	Yes	1-A-4
N1 1-258(C)	Application of antimetabolites for selecting mutants of Blakeslea trispora to enhance yields of beta-carotene by fermentation of cereal grain and other agricultural products.	Chicago, Ill.	Yes	3-B-1
N1 1-259	Exploratory investigations on the conversion of cereal grain carbohydrates to industrial materials through the use of microbial enzyme systems.	Peoria, Ill.	Yes	1-C-3
N1 1-260(C)	Preparation, characterization, and chemical modification of polypeptides derived from cereal proteins to yield products of potential industrial interest as a basis for increasing the utilization of cereal grains.	Chicago, Ill.	Yes	4-B-2
N1 1-261(C)	Establishment of practical conditions for the application of acid-modified flour as a surface-sizing agent for paper on a semicommercial paper machine.	Berlin, N. H.	Yes	4-D-2
N1 1-262(C)	Evaluation of cyanoethylated modified corn starches for application in paper processes as a basis for the development of expanded markets for cereal grain products.	Kalamazoo, Mich.	Yes	1-D-5
N1 1-263	Study of plant-protective and chemical properties of antibiotics produced by fermentation of cereal-based media with streptomycetes and correlation of taxonomic characteristics of antibiotic-producing microorganisms with antibiotic identities.	Peoria, Ill.	Yes	1-C-6
N1 1-264	Engineering studies on modifications of cereal flours and starches to prepare useful polymeric products and to evaluate the products and processes.	Peoria, Ill.	Yes	1-D-5
N1 1-265(Gr)	Basic investigations on the mechanical and viscoelastic properties of corn kernels as influenced by tempering conditions employed in the dry-milling process.	University Park, Pa.	Yes	2-C-2
N1 1-266	Investigations on the application of NMR spectroscopy to grain constituents and derived products to provide information on chemical and molecular structure pertinent to utilization research.	Peoria, Ill.	Yes	1-A-4
N1 1-267(C)	Investigations on the protein content, amino acid composition, and biological feeding value of hybrid grain sorghums and selected milling products as a foundation for improvements in the utilization of grain sorghum in foods and feeds.	Manhattan, Kans.	Yes	9-A-1
N1 1-268(Gr)	Synthesis and degradation of O-glycosides of hydroxy amino acids that form protein to carbohydrate linkages in glycoproteins to provide basic information on the reactions of carbohydrates in proteinaceous compounds and specific information useful for characterizing cereal glycoproteins.	Milwaukee, Wisc.	Yes	4-A-3
N1 1-269(Gr)	Investigations on the principles of disc electrophoresis as a method for large-scale separation of proteins and enzymes.	Manhattan, Kans.	Yes	1-C-3

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-270(Gr)	Basic studies on heat, mass, and momentum transport in cereal starches and flours to provide information for the design of more economical processes for chemical and physical modifications.	Ames, Iowa	Yes	1-A-4
N1 1-271(Gr)	Investigations on amylases from bacteria and on their action patterns and products found on amylolysis of starch and related substrates.	Ames, Iowa	Yes	1-C-3
N1 1-272(Gr)	Basic research on the preparation and characterization of sugars containing carbon bound nitrogen, phosphorus, and sulfur.	Lafayette, Ind.	Yes	1-B-3
N1 1-273(Gr)	Basic studies on the mechanism and effects of chemical cleavage of disulfide bonds in wheat and corn endosperm proteins to provide information for improving the processing and utilization of cereal grains.	Lafayette, Ind.	Yes	1-A-3
N1 1-274(C)	Exploratory studies on the suitability of starch derivatives as protective colloids and binders for use in water-emulsion paints.	Columbus, Ohio	Yes	1-D-4
N1 1-275(C)	Studies on the physical and chemical factors that govern retention and effectiveness of starch xanthates and xanthides by wood pulp to provide information basic to use of these products in papermaking.**	Appleton, Wisc.	Yes	1-B-7
N1 1-276	Exploratory investigations on the enzymatic conversion of cereal starches and glucose to unique glucosides, polyols and isomerization products of glucose as a basis for increasing the industrial utilization of cereal grains.	Peoria, Ill.	Yes	1-C-3
N1 1-277	Study of principles associated with tall fescue which cause toxicity in cattle or limit utilization of the nutrients of this grass.	Peoria, Ill.	Yes	17-A-1
N1 1-278(Gr)	Studies of starch reactions in a dielectric field as a basis for increasing the industrial utilization of cereal starches.**	Pittsburgh, Pa.	Yes	1-B-5
N1 1-279(Gr)	Studies on the amination of starch for the purpose of modifying its chemical and physical properties as a basis for expanded utilization.**	Columbus, Ohio	Yes	1-B-3
N1 1-280	Investigations on the composition and distribution of lipids in the hybrid corn kernel and changes related to storage and artificial drying to provide information basic to the dry- and wet-milling of corn and production of products of maximum value and utility.**	Peoria, Ill.	Yes	3-A-3
N1 1-281	Exploration of microbial spores as fermentative agents for converting cereal grains to industrial products.**	Peoria, Ill.	Yes	1-C-3
N1 1-282(C)	Investigation of typical Aspergilli that may grow on cereal grains to produce toxins, as a basis for improving the production of wholesome foods and feeds.**	Brookings, S. Dak.	Yes	3-B-2
N1 1-283	Studies on copolymerization, addition and replacement reactions of free and potential functional groups of wheat gluten proteins with vinyl monomers and related chemicals as a basis for developing new industrial products from these proteins.**	Peoria, Ill.	Yes	4-B-1

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N1 1-284	Engineering investigations on improved tempering and degerminating procedures for dry milling corn of 15-20 percent moisture content, and development of a flotation separation process and of dehulling equipment for an improved process for manufacturing dry-milled corn products.**	Peoria, Ill.	Yes	2-C-1
N1 1-285	Study of microscopic and ultrastructure of wheat grains and flours and of changes induced by enzymes, moisture, and various treatments to obtain information leading to improved processing methods and new products.**	Peoria, Ill.	Yes	5-A-1
N1 1-286(Gr)	Fine structure of microbial polysaccharide NRRL B-1973 obtained by fermentation of starch-derived sugars.**	Madison, Wisc.	Yes	1-C-5
N1 1-287	Investigations on the dispersion, solubility, and structure of starches from various corn genotypes which contain different proportions of amylose and amylopectin to provide basic information for their utilization.**	Peoria, Ill.	Yes	1-A-3
N1 1-288(Gr)	Studies on the mechanism of hydrolysis of cereal starches by enzymes using Bacillus subtilis amylase and sweet potato beta-amylase as model enzyme systems.**	Fayetteville, Ark.	Yes	1-C-3
N1 1-289(Gr)	The reaction of diepoxides with corn starch and its derived products.**	Tucson, Ariz.	No	
N1 1-291(C)	Studies on the use of starch-derived glycol and glycerol glycosides as protective coatings, plastics, and resins.**	Minneapolis, Minn.	Yes	1-D-4
N1 1-292(C)	Development studies on the use of starch and starch derivatives for the reinforcement of synthetic and natural rubber products.**	Akron, Ohio	Yes	1-B-7
N1 1-293(Gr)	Basic studies on reactions of the helical form of amylose with small molecules.**	Tempe, Ariz.	Yes	1-A-3
N1 1-294	Exploration of methods for the preparation of halogen-substituted derivatives of glucose, starch, and related carbohydrates as a basis for the development of new products for industrial use from cereals.**	Peoria, Ill.	Yes	1-B-3
N1 1-295	Investigations of the amounts, distribution, amino acid composition, and molecular structure of the different proteins of corn grain to provide basic information needed for improving the milling of corn for food, feed, and industrial uses.**	Peoria, Ill.	Yes	3-A-1
N4 2	Soybean and other oilseed utilization investigations--Northern region.			
N4 2-77 (Rev.)	Engineering investigations on the production of cyclic fatty acids from linseed oil.*	Peoria, Ill.	Yes	12-D-1
N4 2-78	Chemical and physical characterization studies on the electrophoretically and chromatographically separated proteins found in defatted soybean meal whey and on their associated non-protein components to provide information basic to improving the industrial and feed value of whey proteins.*	Peoria, Ill.	Yes	11-A-2

*Discontinued during reporting year.
**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
N4 2-83(C)	Studies on the effect of linseed oil coatings on the durability of air-entrained concrete and its resistance to freeze-thaw cycles for evaluating the use of this oil in protecting this type of concrete against deterioration.*	Manhattan, Kans.	Yes	12-D-2
N4 2-84 (Rev.)	Exploratory investigations on products obtained by reacting linseed and soybean oils and their fatty acids with selected polyols and other hydrophilic reagents, and characterization of the products for utility as water-soluble paint vehicles.*	Peoria, Ill.	Yes	12-B-2
N4 2-85	Investigations on the preparation, properties, and reactions of aldehyde oils obtained by the ozonolysis of soybean, linseed and erucic acid oils, as a basis for their increased industrial utilization.	Peoria, Ill.	Yes	10-B-1,2
N4 2-86	Investigations on new polymeric products from aldehydic materials obtained by the ozonization of soybean and linseed oils, as a basis for increased industrial utilization of these oils.	Peoria, Ill.	Yes	10-B-2
N4 2-87	Engineering studies on the production of aldehyde oils from soybean, linseed, and other unsaturated vegetable oils.*	Peoria, Ill.	Yes	10-D-1
N4 2-88	Basic investigations on the chemical reactions of soybean and linseed oils and their fatty acids with ethylene and other commercially available olefinic compounds to produce new products having potential industrial value.	Peoria, Ill.	Yes	12-B-1
N4 2-89	Studies on edible soybean oil: Improvements achieved by mixing soybean oil with other edible oils.*	Peoria, Ill.	Yes	11-B-3
N4 2-90(C)	Investigations on flatus caused by ingestion of soybean foods as related to the development of foreign-type foods to expand export markets.	Urbana, Ill.	Yes	11-C-1
N4 2-91	Preparation of new derivatives from soybean and linseed fatty acids or oils containing vinyl groups capable of polymerizing to form new polymers and copolymers of potential industrial value in coatings and related fields.*	Peoria, Ill.	Yes	12-B-2
N4 2-92(C)	Preparation and evaluation of heterogeneous catalysts for specificity in hydrogenation of linolenate in soybean and linseed oils to increase industrial and food applications.*	Chicago, Ill.	Yes	11-B-2
N4 2-93(C)	Basic investigations on heterogeneous catalysts for the selective hydrogenation of linolenate in soybean oil to provide basic information for increased food applications.	New Brunswick, N. J.	No	
N4 2-94(C)	Investigations on the conversion of aldehyde oils to coating compositions for wood and metal as a basis for developing new industrial products from linseed and soybean oils.	Fargo, N. Dak.	Yes	12-B-2
N4 2-95	Investigations on improving flavor stability of edible soybean oil by concurrent ester interchange of the oil and selective extraction of trilinolenin.*	Peoria, Ill.	No	
N4 2-96 (Rev.)	Relation of minor constituents of soybeans to flavor, flatus, and color of soybean protein food products.	Peoria, Ill.	Yes	11-B-5
N4 2-97(C)	Basic studies on the organo complexes of transition metals as homogeneous catalysts for hydrogenation of soybean oil.	Urbana, Ill.	Yes	11-B-1

*Discontinued during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2-98	Investigations on heat gelation of alcohol-washed soybean protein as a basis for developing new food and industrial uses for this protein.	Peoria, Ill.	Yes	11-A-2
N4 2-99(C)	Studies on the effect of linseed oil coatings on the curing and durability of concrete, and evaluation of selected linseed oil compositions for this potentially new use.	Manhattan, Kans.	Yes	12-D-2
N4 2-100(C)	Investigations on the preparation of copolymers of linseed oil and vinyl monomers suitable for emulsion paints.	Menlo Park, Calif.	Yes	12-B-2
N4 2-101(C)	Investigations on the chemical and physical properties of poly(ester-acetals) and poly(amide-acetals) derived from soybean and linseed oils and of the bonds formed between them and various substrates after crosslinking.	Dedham, Mass.	Yes	10-D-1
N4 2-102	Microbial modification of fatty acids to produce derivative long-chain acids of potential industrial utility.	Peoria, Ill.	Yes	10-C-1
N4 2-103	Basic studies on the water permeability and water sensitivity of linseed oil films, as a basis for increasing the utilization of linseed oil in paints.	Peoria, Ill.	Yes	12-B-3
N4 2-104	Investigations on the effect of emulsion and oil viscosities, and of particle size in oil-in-water emulsions on the film-forming properties of linseed oil, as a basis for increasing the utilization of linseed oil in paints.	Peoria, Ill.	Yes	12-B-3
N4 2-105(C)	Pilot preparation of materials for developmental investigations on aldehyde oils and related products derived from soybean and linseed oils.	Minneapolis, Minn.	Yes	10-D-1
N4 2-106	Investigation of chemical and molecular structure of vegetable oils and their derivatives from soybeans, flax and other commodities of the Northern Region by mass spectrometry to obtain information pertinent to utilization research.	Peoria, Ill.	Yes	11-A-1
N4 2-107	Investigation of selective heterogeneous catalytic hydrogenation to provide information basic to increasing the edible use of soybean oil.	Peoria, Ill.	Yes	11-B-2
N4 2-108	Basic investigations on homogeneous catalysts for selective hydrogenation of soybean and other linolenate oils for use in edible and industrial products.	Peoria, Ill.	Yes	11-B-1
N4 2-109	Evaluation of edible soybean oil products by chemical, physical, and organoleptic methods to provide basic information for increasing applicability in food uses.	Peoria, Ill.	Yes	11-B-3
N4 2-110(CA)	Preparation and evaluation of selected linseed oil derivatives for use in protective coatings to eliminate blistering, prevent yellowing, and improve tint retention.**	Fargo, N. Dak.	Yes	12-B-3
N4 2-111	Exploration of new nitrogen- and sulfur-containing polymers derived from linseed oil to give products suitable for use in protective coatings.**	Peoria, Ill.	Yes	12-B-2
N4 2-112(C)	Investigations on the mechanism of homogeneous catalysis by organometallic complexes for the selective hydrogenation and isomerization of soybean oil.**	Urbana, Ill.	No	

**Initiated during reporting year.

Line Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
N4 2-113	Investigations on a pilot-plant scale of the selective hydrogenation of soybean oil with heterogeneous catalysts to develop large-scale methods for producing flavor-stable, liquid edible oil.**	Peoria, Ill.	No	
NU-0-0-1 (AID)	Engineering development of processes for converting soybeans to high-quality full-fat soybean flours for use in various developing countries of the world.	Peoria, Ill.	Yes	11-E-1
N5 5	New and replacement crops utilization investigations.			
N5 5-15 (Rev.)	Chemical screening to determine the amount and kind of fiber and accompanying constituents in selected plants, as a basis for discovering potential new domestic sources of fiber for pulp and papermaking.	Peoria, Ill.	Yes	13-A-2
N5 5-32 (Rev.)	Chemical survey of seed lipids from uncultivated domestic and foreign plants to discover sources containing economic amounts of industrially valuable constituents.	Peoria, Ill.	Yes	13-A-1
N5 5-33 (Rev.)	Characterization of selected fractions and chemical components of seeds of plant species containing favorable amounts of gross constituents to obtain more specific evaluation of their potential industrial importance than is afforded by screening analyses.	Peoria, Ill.	Yes	13-A-3
N5 5-41	Investigation of selected plants of the Hibiscus genus, with emphasis on kenaf and okra, to evaluate and develop fibrous products from annual plant sources having superiority or specific preferred properties for industrial use.*	Peoria, Ill.	Yes	13-B-1
N5 5-44 (Rev.)	Chemical studies on nitrogenous components, other than enzymes, of crambe and other new oilseed meals as a basis for improved meal utilization for feed and industrial purposes.	Peoria, Ill.	Yes	14-A-1; 16-A-2,3
N5 5-47	Engineering studies on a process for converting Crambe abyssinica seed and closely related new oilseeds into oil and detoxified meal for evaluating the utilization potential of these new oilseed crops.	Peoria, Ill.	Yes	16-B-1
N5 5-48(C)	Investigations on the preparation of omega-amino tridecanoic acid from crambe oil, the production of polyamides from it, and evaluation of the polymers for industrial uses.	Birmingham, Ala.	Yes	15-B-1
N5 5-49	Investigation of the enzyme systems, bitter principles, pigments and other selected constituents of defatted crambe and closely related seed meals considered pertinent to seed processing and meal utilization as feed.	Peoria, Ill.	Yes	16-A-1,3
N5 5-50	Investigations on the chemical modification of crambe oil, and of acids readily derived from it to prepare derivatives or chemical intermediates having properties desirable for industrial use.	Peoria, Ill.	Yes	15-A-1
NU P-1	Pioneering Laboratory for Microbiological Chemistry	Peoria, Ill.	Yes	1-C-7

*Discontinued during reporting year.
**Initiated during reporting year.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Subheading
(10)	Cereal and forage crops.			
UR-A7-(10)-9	Collection and isolation of molds belonging to the order Mucorales, and classification of the isolates, in order to find micro-organisms suitable for fermentative processes of importance in cereal grain utilization.	Allahabad, India	Yes	1-C-1
UR-A7-(10)-10	A study of survival and possible genetic change in industrially useful microorganisms subjected to lyophilization, to obtain basic information needed for the maintenance of culture collections for industrial fermentation of cereal grains.	Allahabad, India	Yes	1-C-1
UR-A7-(10)-20	Investigations on the preparation and characterization of new copolymers of cereal starch with other polysaccharides by heating mixtures in the dry state, to provide basic information for the development of new starch products suited for industrial applications.	Ahmedabad, India	Yes	1-B-7
UR-A7-(10)-25	Investigations on the separation of grain sorghum proteins into homogeneous protein components, to provide basic information for further characterization and application studies.	Bangalore, India	Yes	9-A-2
UR-A7-(10)-75 (Rev.)	Investigation of the distribution of aerobic actinomycetes in India, with particular emphasis on their isolation, characterization, antibiotic production, and preservation, for placement in the Culture Collection of the Agricultural Research Service as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.	Lucknow, India	Yes	1-C-1
UR-A7-(10)-98	Investigations of methods for the chemical preparation and characterization of hydroxyethyl ethers of cereal starches prepared by partial replacement of specific hydroxyl groups to obtain new starch products with improved properties.	Ahmedabad, India	Yes	1-B-7
UR-A7-(10)-111	Studies on the isolation from natural plant gums of aldobi- and aldotriuronic acids to provide reference compounds for structural investigations on microbial polysaccharides of potential industrial significance that are produced from cereal grains.**	Kanpur, India	No	
UR-A10-(10)-27	Studies on the preparation and properties of graft copolymers of starch and dextrin obtained by reaction with vinyl monomers and epoxides, to provide a basis for increased industrial utilization of cereal grains.	Jerusalem, Israel	Yes	1-B-4
UR-A10-(10)-51	Fundamental studies on the mild oxidation of cereal grain starches by selected oxidizing agents for the determination of reaction mechanisms and the physical and chemical properties of modified starches of importance to their production and industrial use.	Jerusalem, Israel	No	
UR-A11-(10)-17	Investigations to discover microorganisms that produce useful quantities of D-tartaric acid, and to explore laboratory processes for its production by fermentation of cereal grain products, as a basis for increasing the utilization of such products.	Tokyo, Japan	Yes	1-C-8

**Activated during reporting year.

PL 480 Research Project Check List -- Reporting Year July 1, 1965 to June 30, 1966 (Cont'd.)

Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-A11-(10)-19	Investigations on the development of a polarographic method for determining aldehyde and ketone carbonyl groups in products derived from cereal grain starches to facilitate the characterization of such polysaccharides as a basis for increasing their utilization.	Sakai, Japan	Yes	1-A-4
UR-A11-(10)-22	Investigations to discover microorganisms that produce useful quantities of mevalonic acid, and to develop an efficient process for the economic production of mevalonic acid by fermentation of cereal grain products, as a basis for increasing the utilization of such products.	Tokyo, Japan	Yes	1-C-8
UR-E4-(10)-2	Search for lytic enzymes of microbial origin with activity on cell walls of bacteria, actinomycetes, molds, and yeasts to provide a basis for the development of new fermentation processes for the increased utilization of cereal grains.	Liege, Belgium	Yes	1-C-3
UR-E9-(10)-37 (Rev.)	Fundamental investigations of the proteolysis-inhibiting effects of cereal flours and starches, and of processing methods for minimizing such effects, to provide a basis for improved quality and increased utilization of cereal products.*	Paris, France	Yes	5-A-4
UR-E9-(10)-40	Investigations of the zein protein of corn: Fractionation and study of rheological and physical-chemical properties, chemical composition and structure, and problems of hydration and gelification of fundamental importance to the technology and industrial utilization of corn proteins.*	Paris, France	Yes	1-A-3
UR-E9-(10)-42	A fundamental investigation of the physico-chemical alterations brought about in starches and their molecular constituents by gamma-radiations, to provide information needed for modification of starch properties and for the treatment of starch-containing products used industrially or in foods.*	Paris, France	No	
UR-E9-(10)-56	Selection and mutation of strains of yeast capable of producing high quantities of sulfur-containing amino acids for use in increasing the efficiency of cereal-grain-based feeds deficient in these amino acids.***	Paris, France	Yes	3-B-4
UR-E15-(10)-21	Investigation of the growth factor (Vitamin B_{12}) of distillers' dried solubles through studies of methods of isolation and purification, mode of formation, and conditions of optimum production by yeast fermentation of cereal grains, to provide basic information for utilizing grains to produce this vitamin.*	Milan, Italy	Yes	3-B-3
UR-E15-(10)-25	Investigations of the reaction of cereal starch dextrins with fatty acid chlorides and fatty amines, and evaluation of the products, to provide information important to increasing the utilization of wheat, corn, and sorghum.*	Bologna, Italy	Yes	1-B-3

*Completed project.
***Terminated prior to completion.

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Subheading
UR-E15-(10)-26	Investigation of the fermentative conversion of glucose to 5-ketogluconic acid through studies of a metabolic pathway in organisms of the Acetobacter genus, to obtain fundamental information for the utilization of grain products in the fermentative production of chemical intermediates.*	Milan, Italy	Yes	1-C-7
UR-E15-(10)-32	Investigations on the conformation of glucopyranose rings in amylose corn starches and in linear and cyclic dextrins prepared from these starches, to provide basic information for the chemical modification of starch-derived products for the development of new uses.	Milan, Italy	Yes	1-A-4
UR-E19-(10)-18	Studies on the preparation of metal alkoxides of starch for use as intermediates in the synthesis of starch derivatives, to provide a basis for increasing the industrial utilization of cereal starches.	Delft, The Netherlands	Yes	1-B-3
UR-E21-(10)-11	Investigations on the fermentative production of itatartaric acid from glucose, sucrose, or molasses to provide new industrial outlets for these agricultural materials.	Lodz, Poland	Yes	1-C-8
UR-E21-(10)-34	Studies on carotene biosynthesis by the mold Blakeslea trispora, with emphasis on factors in spent mycelia of the mold that stimulate carotene production, to increase the yield of β-carotene obtained by fermentation of cereal grains.**	Poznan, Poland	No	
UR-E25-(10)-45	Investigation of the distribution of aerobic actinomycetes in Spain, with particular emphasis on their isolation, characterization, antibiotic production and preservation, for placement in the Culture Collection of the Agricultural Research Service as potential agents for the conversion of farm-produced raw materials to products useful to industry and the consuming public.**	Madrid, Spain	Yes	1-C-1
UR-E29-(10)-37	Studies on the quantitative measurement of properties of wheat kernels that vary significantly during conditioning, as a basis for improved conditioning of wheat for milling by new and improved methods and increased industrial utilization of flour and milled wheat products.*	St. Albans, England	Yes	5-C-3
UR-E29-(10)-51	Investigation of sugars, their phosphate derivatives, and related compounds, as found in molds important to the fermentative conversion of cereal grains to useful products.	Newcastle upon Tyne, England	Yes	1-C-7
UR-E29-(10)-69	Fundamental studies on the nature and specificity of starch- and glycogen-debranching enzymes and the application of these enzymes to a study of the fine structures of amylopectins, amyloses, and glycogens of cereal grains, to provide a basis for increased utilization of cereal grains.	London, England	Yes	1-A-3

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UR-E29-(10)-71	Investigations on the mechanism and structural changes involved in thermal, acid, or alkali degradation of cereal starches, to provide basic information for the development of new starch products suited for industrial applications.	Edinburgh, Scotland	Yes	1-B-5
UR-E30-(10)-1	Studies on the modification of cereal grain starches by physical treatment of granular starch under different conditions of moisture, temperature, and pressure in order to impart new paste properties as a basis for increased utilization of cereal grains.	Ljubljana, Yugoslavia	No	
UR-S3-(10)-11	Preparation of cationic cereal starch derivatives for use in paper and textiles by the introduction of quaternary phosphonium and tertiary sulfonium groups into crosslinked and noncrosslinked starches, to create new markets and expand old markets for starch from cereal grains.	Rio de Janeiro, Brazil	No	
(10,40)	Cereal, forage crops, and oilseeds.			
UR-A11-(10,40)-10	Investigation of crosses of Saccharomyces rouxii isolated from the soybean fermentations, shoyu and miso, and an evaluation of their fermentative abilities in the above fermentation processes, as a basis for increasing the use of soybeans in fermented foods.	Noda-shi, Chiba-ken, Japan	Yes	11-D-3
(40)	Oilseeds			
UR-A6-(40)-1	Investigation of the various processes used in preparing Chinese cheese by the fermentation of soybean curd with Mucor and other fungi as a basis for increasing the foreign utilization of soybeans.	Taipei, Taiwan	Yes	11-D-4
UR-A7-(40)-21	Exploratory investigations of selected hydroxylated derivatives of linseed and safflower oils, to determine the feasibility of producing new industrial products from these oils.	Hyderabad, India	Yes	12-B-5
UR-A7-(40)-95	Laboratory studies on the fermentative production of microbial lipases that are useful in converting vegetable oils to products of higher value as a basis for increasing the utilization of soybean and linseed oils.	Baroda, India	No	
UR-A10-(40)-17	Fundamental investigations of complexes formed by soybean proteins with other meal constituents, to provide information for applied studies on expanded utilization of soybean oil meal.	Rehovot, Israel	Yes	11-A-3
UR-A10-(40)-18	Investigations of soybean saponins as related to the processing of petroleum ether-extracted meal for feed and to the preparation of soy foods, to provide information basic to improving the nutritional value of soybean protein products.*	Rehovot, Israel	Yes	11-A-3
UR-A10-(40)-20	Laboratory investigations on miso-type food products by fermentation of soybean meal products and cereal grains for use in Israeli foods.	Ramat Gan, Israel	Yes	11-D-3

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UR-A10-(40)-30	Investigations of the effect of processing conditions on the yield and quality of isolated soybean protein for use in Israeli-type foods, as a contribution to expanded utilization of soybeans.	Haifa, Israel	Yes	11-E-2
UR-A11-(40)-2	Evaluation of dehulled soybean grits from United States varieties for making miso, to increase soybean utilization in Japan.	Tokyo, Japan	Yes	11-D-3
UR-A11-(40)-5	Investigation of the partial hydrogenation of soybean oil, to produce a stable liquid oil with improved properties for use in Japanese foods.	Kawagoe, Saitama-ken, Japan	No	
UR-A11-(40)-8	Isolation and determination of the flavor components of enzymatically or chemically modified soybean meal and proteins, and elucidation of their chemical and physical properties, to provide information basic to improving the flavor and thus increasing the utilization of soybeans.	Tokyo, Japan	Yes	11-B-5
UR-A11-(40)-11	Evaluation of United States soybean varieties as a material for producing fresh tofu to increase utilization in Japan.	Tokyo, Japan	Yes	11-D-2
UR-A11-(40)-12	A chromatographic study of the sugars and oligosaccharides in soybeans to provide information needed to improve processing of fat-free soybean meal for foods and feeds, thereby contributing to its expanded utilization.*	Takamatsu, Japan	Yes	11-A-3
UR-A11-(40)-13	Isolation, characterization, and quantitative determination of the sterols in soybeans to provide basic information for the evaluation and improvement of soybean meal and soybean products as foods and feeds.	Tokyo, Japan	Yes	11-A-3
UR-A11-(40)-14	Fundamental studies on color reversion of edible soybean oil to obtain information on its cause and prevention, as a means of increasing the utilization of soybean oil for food purposes in Japan.	Tokyo, Japan	Yes	11-C-2
UR-A11-(40)-21	Investigations on comparative production of shoyu (soy sauce) from defatted soybean meals obtained from United States and Japanese soybeans and processed by United States and Japanese methods, to provide data for the increased use of United States beans.**	Tokyo, Japan	No	
UR-A11-(40)-26	Basic investigations on the development of foods from enzymatically treated soybean protein concentrates, to increase the use of United States soybeans in Japan.**	Tokyo, Japan	No	
UR-A11-(40)-31	Studies on enzymatic hydrolysis of soybean oligosaccharides to provide information basic to increasing the utilization of soybean food and feed products.**	Takamatsu, Japan	Yes	11-A-3
UR-E15-(40)-8	An investigation of the minor constituents of linseed oil and their effect on the ability of linseed oil films to spread and adhere to surfaces, as a contribution to the expansion of markets for linseed oil.*	Milan, Italy	Yes	12-A-2

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UR-E15-(40)-9	Investigations of the controlled thermal polymerization of soybean and linseed oils, and of the separation and characterization of the reaction products, in order to obtain information useful in expanding and improving the industrial applicability of these oils.*	Milan, Italy	Yes	12-B-4
UR-E15-(40)-10	Investigations of the effect of metallic catalysts and physical conditions on oxidative cleavage products produced in the autoxidation of polyunsaturated fatty acids, to provide basic information for applied research on the production of new industrial chemicals from soybean and linseed oils.*	Milan, Italy	Yes	10-B-4
UR-E15-(40)-46	Studies on the effect of stereospecific polymerization catalysts on fatty esters from soybean and linseed oils, to provide a basis for increasing the industrial utilization of these vegetable oils.	Milan, Italy	Yes	12-B-4
UR-E15-(40)-48	Synthesis and use of lipid-soluble metal chelates of Schiff bases as catalysts for the selective hydrogenation of soybean oil, to provide a basis for improving its flavor stability for edible use.	Milan, Italy	Yes	11-B-1
UR-E21-(40)-6	Chromatographic determination of the glyceride composition of selected erucic-acid containing oils, to provide basic information important to their utilization.	Warsaw, Poland	Yes	13-A-3
UR-E21-(40)-8	Investigation of the possible role of sterols in the development of flavors and odors in soybean oil through studies of sterol transformations during processing, in order to increase the utilization of soybeans in food.*	Gdansk, Poland	Yes	11-B-4
UR-E25-(40)-29	Improvement of the frying quality of soybean oil through studies of the influence of processing factors and oil modifications on surface tension, interfacial tension, viscosity, and other physical properties concerned with its penetration into fried foods, to provide information for increased use in the preparation of Spanish foods.	Granada, Spain	Yes	11-B-3
UR-E26-(40)-3	Compositional investigations of Swedish Cruciferae (mustard family) seeds to find strains with maximum erucic acid content in their oils and minimum content of glucosidic precursors of isothiocyanates and thiooxazolidones in their meals, to provide a basis for their utilization as industrial oilseeds in the United States.	Svalof, Sweden	Yes	13-A-1
UR-E29-(40)-49	Investigation of the reactions of unsaturated fatty acids and their derivatives in molten alkali, to discover new chemical intermediates important to the increased utilization of soybean and linseed oils.*	London, England	Yes	10-B-4
UR-E29-(40)-50	A quantitative study of the polysaccharides in fat-free soybean meal to provide information needed to improve the processing of meal for foods and feeds, thereby contributing to its expanded utilization.*	Edinburgh, Scotland	Yes	11-A-3

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(50) UR-E15-(50)-29 (Rev.)	Sugar and miscellaneous crops. Preparation and characterization of dextran derivatives, and investigations of their interactions and binding, to provide basic information for increasing the utilization of sugar.	Rome, Italy	No	